PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6: A61K 35/12, C07H 17/00, C07K 1/00, 14/00, 16/00, C12N 1/00, 5/00, 15/00, C12Q 1/00, 1/68, G01N 33/53

A1

(11) International Publication Number: WO 95/19779

(43) International Publication Date: 27 July 1995 (27.07.95)

(21) International Application Number: PCT/US95/00825

(22) International Filing Date: 20 January 1995 (20.01.95)

(30) Priority Data:
185,432 21 January 1994 (21.01.94) US

(71) Applicant: YALE UNIVERSITY [US/US]; 451 College Street, New Haven, CT 06520 (US).

(72) Inventors: ARTAVANIS-TSAKONAS, Spyridon; 192 Ridgewood Avenue, Hamden, CT 06517 (US). BUSSEAU, Isabelle; 154, rue Charles-de-Gaulle, F-91440 Bures-sur-Yvette (FR). DIEDERICH, Robert, J.; 430 Fountain Street, New Haven, CT 06515 (US). XU, Tian; 170 Marshall Avenue, Guilford, CT 06437 (US). MATSUNO, Kenji; 789 Orange Street, 3rd floor, New Haven, CT 06510 (US).

(74) Agents: MISROCK, S., Leslie et al.; Pennie & Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US).

(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, JP, KE, KG, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: *DELTEX* PROTEINS, NUCLEIC ACIDS, AND ANTIBODIES, AND RELATED METHODS AND COMPOSITIONS

(57) Abstract

The present invention relates to nucleotide sequences of *deltex* genes, and amino acid sequences of the encoded *deltex* proteins. The invention further relates to fragments and other derivatives, and analogs, of *deltex* proteins, as well as antibodies thereto. Nucleic acids encoding such fragments or derivatives are also within the scope of the invention. Production of the foregoing proteins and derivatives, *e.g.*, by recombinant methods, is provided. In a specific embodiment, the invention relates to *Drosophila deltex* nucleic acids and proteins. In another embodiment, the invention relates to human *deltex* nucleic acids and proteins. In specific embodiments, the invention relates to *deltex* protein derivatives and analogs of the invention which are functionally active, or which comprise one or more domains of a *deltex* protein, including but not limited to the Glu-rich clusters, SH3 binding domains, domains which mediate binding to Notch or to a Notch derivative containing Notch cdc10/SW16/ankyrin ("ANK") repeats, domains which mediate binding to a second *deltex* protein, or any combination of the foregoing. The present invention also relates to therapeutic and diagnostic methods and compositions based on *deltex* proteins, nucleic acids, and antibodies.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

DELTEX PROTEINS, NUCLEIC ACIDS, AND ANTIBODIES, AND RELATED METHODS AND COMPOSITIONS

This invention was made with government support under grant numbers GM29093 and NS26084 awarded by the National Institutes of Health. The government has certain rights in the invention.

1. INTRODUCTION

The present invention relates to *deltex* genes and their encoded protein products, as well as derivatives and analogs thereof. The invention further relates to production of deltex proteins, derivatives and antibodies. Related therapeutic compositions and methods of therapy and diagnosis are also provided.

2. BACKGROUND OF THE INVENTION

In *Drosophila melanogaster*, the so called "*Notch* group" of genes has been implicated in events crucial for the correct developmental choices of a wide variety of precursor cells (for review, see Fortini and Artavanis-Tsakonas, 1993, Cell 75:1245-1247; Artavanis-Tsakonas and Simpson, 1991, Trends Genet. 7:403-408). The accumulated genetic and molecular studies suggest that these genes encode elements of a cell communication mechanism which includes cell surface, cytoplasmic, and nuclear components.

Very little is known about the mechanisms underlying cell fate choices in higher organisms such as vertebrates; a knowledge of such mechanisms could provide insights into pathologies associated with abnormal differentiation events. Thus, a need exists in the art to obtain and characterize the human members of the "Notch group" of genes, including deltex, since these genes appear to play crucial roles in the determination of cell fate.

Numerous developmental genetic studies in recent years have shown that the *Notch* locus plays a central role in regulative events influencing cell fate decisions in *Drosophila* in a very broad spectrum of developing tissues (reviewed in Artavanis-Tsakonas and Simpson, 1991, Trends Genet. 7:403-408;

and in Artavanis-Tsakonas et al., 1991, Ann. Rev. Cell Biol. 7:427-452). This pleiotropy of *Notch* function is revealed by mutations affecting all stages of development and a variety of tissues (*e.g.*, Welshons, 1965, Science 150:1122-1229; Welshons, 1971, Genetics 68:259-268; Shellenbarger and Mohler, 1978, Dev Biol. 62:432-446). A dramatic illustration of *Notch* function is seen in the development of the embryonic nervous system, whereby loss of function mutations cause the misrouting of epithelial precursor cells into a neural developmental pathway and result in what has been termed a 'neurogenic' phenotype (Poulson, 1937, Proc. Natl. Acad. Sci. USA, 23:133-137; Lehman et al., 1983, Roux's Arch. Dev. Biol. 192:62-74).

In attempts to understand the molecular contexts by which the Notch protein communicates signals from the cell surface to the nucleus to effect changes in cell fate, genetic means have been used to identify loci that interact phenotypically with various *Notch* alleles. These genetic studies led to the definition of a small group of interacting loci, which has been operationally termed the 'Notch group' (Artavanis-Tsakonas and Simpson, 1991, Trends Genet. 7:403-408). The other members of the *Notch* group are *deltex* (Xu and Artavanis-Tsakonas, 1991, Genetics 126:665-677), *Enhancer of (split)* [*E(spl)*] (Knust et al., 1987, EMBO J. 6:4113-4123; Hartley et al., 1988, Cell 55:785-795; Preiss et al., 1988, EMBO J. 7:3917-3927; Klambt et al., 1989, EMBO J. 8:203-210), and *mastermind* (*mam*) (Smoller et al., 1990, Genes Dev. 4:1688-1700). *mastermind* and *Enhancer of (split)* encode nuclear proteins (Smoller et al., 1990, Genes Dev. 4:1688-1700; Delidakis et al., 1991, Genetics 129:803-823). *deltex* mutations suppress the pupal lethality conferred by certain heteroallelic combinations of the *Abruptex* class of *Notch* alleles (Xu et al., 1990, Genes Dev. 4:464-475). From this same genetic screen, the genes *Delta* and *mastermind* were also identified, both of which belong to the same 'neurogenic' class of genes as *Notch* because of the similar mutant phenotypes they produce. Moreover, subsequent analysis has shown that alleles of *deltex* exhibit genetic interactions with those of *Delta* and *mastermind*, a further suggestion of functional

links among these loci (Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677).

The manner by which *Notch* is thought to influence determinative events is indirect, that is, not through the direct specification of cellular fates. Instead, recent experimental studies (Coffman et al, 1993, Cell 73:659-671; Fortini et al, Nature, in press) indicate that *Notch* activity delays differentiation, and in this manner renders precursor cells competent to receive and/or interpret any number of specific developmental cues (Cagan and Ready, 1989, Genes Dev. 3:1099-1112). In loss of function mutants, this inhibition is lost and cells assume default pathways of differentiation. For example, during the development of the *Drosophila* nervous system, cells that normally would become epidermis instead adopt a neural fate in the absence of *Notch* function. However, a salient feature of *Notch* activity is its pleiotropy. *Notch* is required for the proper specification of many other cell types, including those of the compound eye (Cagan and Ready, 1989, Genes Dev. 3:1099-1112), ovary (Ruohola et al., 1991, Cell 66:433-449; Xu et al., 1992, Development 115:913-922), and mesoderm (Corbin et al., 1991, Cell 67:311-323). Similarly, the widespread expression patterns exhibited by vertebrate *Notch* cognates suggest also a broad-based functional role in these species (Coffman et al, 1993, Cell 73:659-671; Coffman et al., 1990, Science 249:1438-1441; Weinmaster et al., 1991, Development 113:199-205; Weinmaster et al., 1992, Development 116:931-941; Kopan and Weintraub, 1993, J. Cell Biol. 121:631-641; Franco del Amo et al., 1992, Development 115:737-744; Ellisen et al., 1991, Cell 66:649-661; Stifani et al., 1992, Nature Genetics 2:119-127).

Notch homologs have been isolated from a variety of vertebrate species and have been shown to be remarkably similar to their *Drosophila* counterpart in terms of structure, expression pattern and ligand binding properties (Rebay et al., 1991, Cell 67:687-699; Coffman et al., 1990, Science 249:1438-1441; Ellisen et al, 1991, Cell 66:649-661; Weinmaster et al., 1991, Development 113:199-205). Two human *Notch* homologs have been isolated (PCT Publication No. WO 92/19737 dated November 12, 1992), termed hN and

TAN-1. A human *Notch* (*TAN-1*) malfunction has been associated with a lymphatic cancer (Ellisen et al., 1991, Cell 66:649-661).

Notch encodes a large, structurally-complex transmembrane protein, consistent with an involvement in cell-cell communication (Wharton et al., 1985, Cell 43:567-581; Kidd et al., 1986, Mol. Cell. Biol. 6:3094-3108). *Notch* has an extracellular domain containing 36 tandem EGF-like repeats and 3 *Notch/lin12* repeats. The intracellular domain bears several common structural motifs including 6 *cdc10/SW16/ankyrin* repeats ("ANK" repeats) (Lux et al., 1990, Nature 344:36-42; Breeden and Nasmyth, 1987, Nature 329:651-654; Michaely and Bennett, 1992, Trends Cell Biol. 2:127-129; Blank et al., 1992, Trends Biochem. Sci. 17:135-140; Bennett, 1992, J. Biol. Chem. 267:8703-8706)), a polyglutamine stretch known as 'opa', and a PEST motif (Stifani et al., 1992, Nature Genetics 2:119-127). The remarkable degree to which these motifs have been conserved in homologs isolated from mice (Weinmaster et al., 1991, Development 113:199-205; Weinmaster et al., 1992, Development 116:931-941; Kopan and Weintraub, 1993, J. Cell Biol. 121:631-641), rats (Kopan and Weintraub, 1993, J. Cell Biol. 121:631-641; Franco del Amo et al., 1993, Genomics 15:259-264), humans (Ellisen et al., 1991, Cell 66:649-661; Stifani et al., 1992, Nature Genetics 2:119-127; PCT Publication No. WO 92/19737 dated November 12, 1992), and *Xenopus* (Coffman et al, 1993, Cell 73:659-671; Coffman et al., 1990, Science 249:1438-1441) implies that they will have a common biochemical mode of action. In particular, ANK repeats, which constitute the most conserved region (~70% amino acid identity) between Notch and its vertebrate counterparts (Stifani et al., 1992, Nature Genetics 2:119-127), are thought to mediate protein-protein interactions among diverse groups of proteins, including those involved in signal transduction processes and cytoskeletal interactions (Lux et al., 1990, Nature 344:36-42; Breeden and Nasmyth, 1987, Nature 329:651-654; Michaely and Bennett, 1992, Trends Cell Biol. 2:127-129; Blank et al., 1992, Trends Biochem. Sci. 17:135-140; Bennett, 1992, J. Biol. Chem. 267:8703-8706). Indeed, Rebay et al. (1993, Cell 74:319-329) have recently demonstrated that the ANK repeats are crucial for Notch-mediated

signaling events. Both EGF-like repeats and ankyrin motifs are found in a variety of proteins known to interact with other protein molecules. Indeed, evidence has shown a direct interaction between Notch and the products of the *Delta* and *Serrate* loci, which also encode transmembrane proteins containing EGF-like repeats (Fehon et al., 1990, Cell 61:523-534; Rebay et al., 1991, Cell 67:687-699). Despite the evidence to suggest that Notch functions as a cell surface receptor, few biochemical details of Notch function are known.

It has been demonstrated that dominant 'activated' phenotypes result from *in vivo* overexpression of a Notch protein lacking most extracellular, ligand-binding sequences, while 'dominant-negative' phenotypes result from overexpression of a protein lacking most intracellular sequences (Rebay et al., 1993, Cell 74:319-329). Despite this evidence further implicating Notch as a cell surface receptor of extracellular signals, the intracellular nature of the signal transduction cascade is unknown.

Citation of a reference herein shall not be construed as an admission that such reference is prior art to the present invention.

3. SUMMARY OF THE INVENTION

The present invention relates to nucleotide sequences of *deltex* genes, and amino acid sequences of the encoded deltex proteins. The invention further relates to fragments and other derivatives, and analogs, of deltex proteins, as well as antibodies thereto. Nucleic acids encoding such fragments or derivatives are also within the scope of the invention. Production of the foregoing proteins and derivatives, *e.g.*, by recombinant methods, is provided.

In a specific embodiment, the invention relates to *Drosophila deltex* nucleic acids and proteins. In another embodiment, the invention relates to human *deltex* nucleic acids and proteins.

In specific embodiments, the invention relates to deltex protein derivatives and analogs of the invention which are functionally active, or which comprise one or more domains of a deltex protein, including but not limited to

the Glu-rich clusters, SH3 binding domains, domains which mediate binding to Notch or to a Notch derivative containing Notch cdc10/SW16/ankyrin ("ANK") repeats, or any combination of the foregoing.

The present invention also relates to therapeutic and diagnostic methods and compositions based on deltex proteins and nucleic acids. The invention provides for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: deltex proteins and analogs and derivatives (including fragments) thereof; antibodies thereto; nucleic acids encoding the deltex proteins, analogs, or derivatives; and deltex antisense nucleic acids. In a preferred embodiment, a Therapeutic of the invention is administered to treat a cancerous condition, or to prevent progression from a pre-neoplastic or non-malignant state into a neoplastic or a malignant state. In other specific embodiments, a Therapeutic of the invention is administered to treat a nervous system disorder or to promote tissue regeneration and repair.

In one embodiment, Therapeutics which antagonize, or inhibit, Notch and/or deltex function (hereinafter "Antagonist Therapeutics") are administered for therapeutic effect. In another embodiment, Therapeutics which promote Notch and/or deltex function (hereinafter "Agonist Therapeutics") are administered for therapeutic effect.

Disorders of cell fate, in particular hyperproliferative (*e.g.*, cancer) or hypoproliferative disorders, involving aberrant or undesirable levels of expression or activity or localization of Notch and/or deltex protein can be diagnosed by detecting such levels, as described more fully *infra*.

In a preferred aspect, a Therapeutic of the invention is a protein consisting of at least a fragment (termed herein "adhesive fragment") of deltex which mediates binding to a Notch protein or a fragment thereof.

4. DESCRIPTION OF THE FIGURES

Figure 1. Map of cloned genomic sequences and alignment of *deltex* cDNA clones. A). Coordinates are indicated in kilobasepairs, 0

corresponding to the P element insertion site of dx^P . The positions of *Eco*RI (R) and *Sal*I (S) restriction sites are shown. Recombinant phages λA25 and λA28 derive from dx^P genomic DNA library (see Section 6.3). Wild-type Canton S clones are represented by λI1, λI2 and λI3. DNA fragments cloned into the Bluescript plasmid vector are denoted with the prefix pd. **B**). Detail of genomic interval -5 to +6.5. *Xba*I (X) restriction sites are also shown. Below the map, open bars represent the cDNAs cBE30 and cBE8; thin lines delineate intronic sequences. *deltex* mRNA sequences represented by the cDNAs are shown as a solid line; broken line depicts the remaining 5' end of the mRNA as inferred from genomic sequencing. Within schematic of the predicted protein, black boxes denote relative position of *opa* repeats. Stippled bar to left of *deltex* transcription unit indicates genomic fragments that hybridize to partial cDNAs representing a ribosomal protein gene.

Figure 2. Developmental Northern blot. Each lane contains 5 μg size-fractionated poly $(A)^+$ RNA isolated from developmentally staged animals. Left: embryonic mRNA, hours (h) after egg laying; (L1-3) first, second and third larval instars. Right: early-(E) and late (L)-staged pupae (P) and adult male and female mRNA. Length of *deltex* mRNA is indicated in kilobases (kb). Band of *deltex* hybridization present in adult male lane is due to contaminating female flies. Pattern of actin 5c mRNA accumulation is shown as control.

Figure 3. Composite nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of the *deltex* locus. DNA sequences in uppercase letters are derived from the cDNAs cBE30 (nucleotide 1 to 1299) and cBE8 (nucleotides 1116 to 3764); lowercase letters refer to genomic sequence from the dx^P strain. The predicted amino acid sequence is depicted below the DNA sequence, with the *opa* repeats shown in bold letters. Numbers along left margin refer to the cDNA sequence; numbers on the right refer to the predicted amino acid sequence. Duplicated nucleotides resulting from insertion of the P element of dx^P are shown boxed. A potential polyadenylation signal is underlined twice; a stretch of 17 A's is found in cDNA cBE8 just after nucleotide 3764 (the last uppercase letter). 5' to the cDNA sequence, two groups of seven

nucleotides (underlined) are homologous to a transcription initiation consensus sequence.

Figure 4. Subcellular pattern of induced deltex protein expression. Confocal microscope images of a stage-10 embryo (lateral view: anterior is to the left ; dorsal is up) after immunofluorescent staining with monoclonal antibody C645.17A. Embryo contains a transposon (line $142A^{D}$) of *deltex* coding sequences under the control of the *Drosophila Hsp*70 promoter and was stained after heat shock induction (see Section 6.3). Upper right: whole embryo; lower left: high magnification of same image detailing ventral region.

Figure 5. The effects of the *Su(dx)* mutations on various *deltex* alleles are summarized. The top row indicates the genetic constitution of the second chromosome and the left column indicates the genetic makeup of the X chromosome in the male. (+++): the *deltex* wing and ocellar phenotypes are completely suppressed. (++): the *deltex* wing and ocellar phenotypes are almost completely suppressed; only a trace of extra wing vein material is present at the margin of the fifth longitudinal veins. (+): the *deltex* wing and ocellar phenotypes are partially suppressed.

Figure 6. Suppression of *deltex* wing phenotypes by the *Su(dx)* mutations. a). Wing of *y* dx^{ENU} sn^{3}/Y male: note notches and extra vein material. b). Wing of *y* dx^{ENU} sn^{3}/Y; $Su(dx)^{w}/+$ male: the dx^{ENU} wing phenotype is completely suppressed by one copy of the $Su(dx)^{w}$ mutation. c). Wing of $dx^{SM}t^{2}$ *v/Y* male: notches and extra vein material are evident along the veins. d). Wing of $dx^{SM}t^{2}$ *v/Y*; $Su(dx)^{w}/+$ male: the phenotype of this strong *dx* allele is completely suppressed by one copy of the $Su(dx)^{w}$ mutation, but is only partially suppressed by the $Su(dx)^{2}$ mutation. e). Wing of dx^{P} sn^{3}/Y male: this weak allele displays a mild extra-vein-material phenotype on the ends of the longitudinal veins. f). Wing of dx^{P} sn^{3}/Y; $Su(dx)^{w}/+$ male: the phenotype of the dx^{P} mutation is only partially suppressed by $Su(dx)^{w}$ (and also by the other two *Su(dx)* mutations).

Figure 7. a).Wing of *nd* dx^{ENU}/Y fly. Note the severe *deltex* wing-notching and the suppressed *Abruptex* gapped-vein phenotype. b). Wing of

Ax^{E2} dx^{ENU}/Y; Su(dx)/+ fly. Note the suppression of the *deltex* phenotype and the reemergence of the *Abruptex* gapped-vein phenotype. c). Dorsal view of $Ax^{E2}sn^{3}$/Y; $Su(dx)^{sp}$/+ fly. d). Dorsal view of Ax^{E2} dx^{ENU}/Y; $Su(dx)^{sp}$/+ fly. Arrow points to region exhibiting a severe loss of micro- and macro-chaetae.

Figure 8. deltex interacts with Notch cdc10/SW16/ankyrin ("ANK") repeats. Confocal microscope images of *Drosophila* S2 cells (a-d and f-l) and distal portion of imaginal wing disc (e) are presented as split images (except k) with Notch expression shown in green and deltex in red. For S2 cells, each panel represents a co-transfection experiment involving the *deltex* expression plasmid pCaSpeR hs-dx (see Section 6) and each of the expression constructs depicted in Fig. 9 or described elsewhere: a and b, pMtNcDNA (full-length Notch; Rebay et al., 1993, Cell 74:319-329); c, pMTDl1 (Delta; Fehon et al., 1990, Cell 61:523-534); d, Ser-mtn (Serrate; Rebay et al., 1991, Cell 67:687-699); f, pMTΔB-S; g, pMTΔS-S; h, pMTECN; i, pMTΔcdc10; j and k, pMTDl/NANK; l, pMTDl/CANK. Cell surface 'capping' was induced (except in b) as described (Fehon et al., 1990, Cell 61:523-534; Rebay et al., 1991, Cell 67:687-699). Immunofluorescent labeling of cells was performed as described (Fehon et al., 1990, Cell 61:523-534) using mouse anti-Notch monoclonal antibody (line C458.2H) specific for the extracellular domain and rat anti-deltex polyclonal or monoclonal (line C645.17A) antibodies (see Section 6) directed against the entire coding region. Extraneous cytoplasmic Notch expression was minimized by reacting cells with anti-Notch antibody prior to cell permeabilization and incubation with anti-deltex antibodies. Delta and Serrate expression (c and d) are viewed indirectly through their co-capping with Notch. In some instances, aggregated cell partners were torn apart during manipulation. The diameter of S2 cells is 7-10 microns.

Figure 9. Plasmid expression constructs used in S2 cell transfections. a, Notch intracellular domain (top; drawn to scale); extracellular domain is not shown but is intact (TM, transmembrane region). Structural motifs and restriction sites used to generate deletion constructs (black bars) are indicated. Deleted amino acids are presented in parentheses (numbering from Wharton et

al., 1985, Cell 43:567-581). b, Intracellular domains of Delta-based expression constructs (same scale as in a). *Nde*I site adjacent to translational stop codon was used as insertion site for Notch- (pMTDI/NANK) and cactus- (pMTDI/CANK) ANK repeat coding sequences. Results of deltex binding are summarized. c, Immunoblot analysis of S2 cell lysates after transfection and expression of plasmid constructs depicted in b (NT, non-transfected cells). Integrity of other expression constructs (a) was also confirmed by immunoblot analysis (not shown).

Figure 10. Direct Notch/deltex interaction in yeast. Each column lists *Drosophila* genes that were fused to sequences encoding a LexA DNA-binding domain or an acidic transcription activation domain. These were co-transformed into yeast containing a *LexA operator-lacZ* reporter gene plasmid (pSH18-34; S. Hanes and R. Brent, unpublished) and were plated onto glucose- and galactose-Ura$^-$ His$^-$ Trp$^-$ X-gal-indicator plates. Three independent yeast isolates are shown for each experiment. Only transformations involving *LexA-Notch ICN1* and *deltex-ACT* resulted in detectable β-galactosidase activity.

Figure 11. Intragenic suppressor mutation maps within fifth Notch ANK repeat. a, Diagram of Notch protein. The *su42c* mutation was induced in flies containing the Ax^{9B2} mutation (Xu et al., 1990, Genes Dev. 4:464-475). b, DNA sequencing gels showing C to T transition in Ax^{9B2} *su42c* genomic DNA. This residue is unaffected (*i.e.*, wild-type) in the parental Ax^{9B2} DNA. c, Amino acid sequence (single letter code) (SEQ ID NO:22) of fifth ANK repeat showing alanine to valine substitution resulting from the *su42c* mutation (numbering based on Wharton et al., 1985, Cell 43:567-581).

Figure 12. Nucleotide sequence (SEQ ID NO:3) and deduced amino acid sequence (SEQ ID NO:4) of deltex cDNA.

Figure 13. Schematic diagram of deltex fragments mediating deltex-deltex interactions.

Figure 14. Amino acid sequence of *Drosophila* deltex (SEQ ID NO:2; SEQ ID NO:4) and designated fragments implicated in protein-protein interactions. Fragments A-D (SEQ ID NOS:5-8, respectively) are shown.

Figure 15. Schematic diagram of the deltex and Notch fragments mediating deltex-Notch interactions.

Figure 16. Putative SH3-binding domains in *Drosophila* (fly). The sequence of the mouse GAP SH3-binding domain (SEQ ID NO:9) is shown, with the putative SH3 binding domains of the *Drosophila* proteins deltex (SEQ ID NO:10 and SEQ ID NO:11), son of Sevenless (SEQ ID NO:12 and SEQ ID NO:13), hairless (SEQ ID NO:14), and disabled (SEQ ID NO:15).

Figure 17. Aligned amino acid sequences of Notch proteins of various species. humN: the human Notch protein encoded by the hN homolog (SEQ ID NO:16). TAN-1: the human Notch protein encoded by the TAN-1 homolog (SEQ ID NO:17) (the sequence shown is derived partly from work by Artavanis-Tsakonas et al. (see PCT Publication No. WO 92/19737 dated November 12, 1992) and partly from the TAN-1 sequence as published by Ellisen et al., 1991, Cell 66:649-661); Xen N: Xenopus Notch protein (Coffman et al., 1990, Science 249:1438-1441 (SEQ ID NO:18)). Dros N: Drosophila Notch protein (Wharton et al., 1985, Cell 43:567-581 (SEQ ID NO:19)). Structural domains are indicated.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to nucleotide sequences of deltex genes, and amino acid sequences of their encoded deltex proteins. The invention further relates to fragments and other derivatives, and analogs, of deltex proteins. Nucleic acids encoding such fragments or derivatives are also within the scope of the invention. Production of the foregoing proteins and derivatives, *e.g.*, by recombinant methods, is provided.

In particular, the invention relates to the *Drosophila* deltex gene and protein. In another embodiment, the invention relates to the human deltex gene and protein.

The invention also relates to deltex protein derivatives and analogs of the invention which are functionally active, *i.e.*, they are capable of displaying one or more known functional activities associated with a full-length (wild-type)

deltex protein. Such functional activities include but are not limited to antigenicity [ability to bind (or compete with a deltex protein for binding) to an anti-deltex protein antibody], immunogenicity (ability to generate antibody which binds to a deltex protein), ability to bind (or compete with a deltex protein for binding) to Notch or a second deltex protein or other proteins or fragments thereof, ability to bind (or compete with a deltex protein for binding) to a receptor or ligand for a deltex protein.

The invention further relates to fragments (and derivatives and analogs thereof) of a deltex protein which comprise one or more domains of a deltex protein (see *infra*), including but not limited to the Glu-rich clusters, SH3 binding domains, domains which mediate binding to Notch (or a derivative thereof containing the Notch ANK repeats) or to a second deltex molecule or fragment thereof, or any combination of the foregoing.

Antibodies to deltex proteins, their derivatives and analogs, are additionally provided.

deltex plays a critical role in development and other physiological processes, in particular, in the signaling pathway of Notch which is involved in cell fate (differentiation) determination. Our results presented by way of example below indicate that deltex mediates the intracellular portion of the signal transduction cascade involved in Notch function. As described therein (see Section 6), our results show that deltex is localized within the cytoplasm, that it is a protein of unique sequence, and that it displays direct molecular interaction with the Notch intracellular ANK repeats, motifs shared by many proteins and implicated in protein-protein interactions (Lux et al., 1990, Nature 344:36-42, Thompson et al., 1991, Science 253:762-768, reviewed in Bennett, 1992, J. Biol. Chem. 267:8703-8706, Blank et al., 1992, Trends Biochem. Sci. 17:135-140, Rebay et al., 1993, Cell 74:319-329). Moreover, an *in vivo* functional analysis of various truncated forms of Notch has implicated these ANK repeats in downstream signaling events (Rebay et al., 1993, Cell, 74:319-329). Furthermore, deltex displays genetic interactions with Notch and Delta, both transmembrane proteins, and with mastermind, a nuclear localized protein

(Smoller et al., 1990, Genes Dev. 4:1688-1700). This makes deltex the first identified cytoplasmic component of the *Notch* group of interacting loci. We further examined the functional characteristics of *deltex* by analyzing its genetic interactions with another locus, *Suppressor of deltex* [*Su(dx)*]. This analysis implicates *Suppressor of deltex* in the genetic circuitry of *Notch* and suggests a three way interaction between *Su(dx)*, *deltex* and *Notch*.

The deltex nucleic acid and amino acid sequences and antibodies thereto of the invention can be used for the detection and quantitation of deltex mRNA and protein, to study expression thereof, to produce deltex proteins, fragments and other derivatives, and analogs thereof, in the study, assay, and manipulation of differentiation and other physiological processes, and are of therapeutic and diagnostic use, as described *infra*. The deltex nucleic acids and antibodies can also be used to clone deltex homologs of other species, as described *infra*. Such deltex homologs are expected to exhibit significant homology to each other, and encode proteins which exhibit the ability to bind to a Notch protein.

The present invention also relates to therapeutic and diagnostic methods and compositions based on deltex proteins and nucleic acids. The invention provides for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: deltex proteins and analogs and derivatives (including fragments) thereof; antibodies thereto; nucleic acids encoding the deltex proteins, analogs, or derivatives; and *deltex* antisense nucleic acids. In a preferred embodiment, a Therapeutic of the invention is administered to treat a cancerous condition, or to prevent progression from a pre-neoplastic or non-malignant state into a neoplastic or a malignant state. In other specific embodiments, a Therapeutic of the invention is administered to treat a nervous system disorder or to promote tissue regeneration and repair.

In one embodiment, Therapeutics which antagonize, or inhibit, Notch and/or deltex function (hereinafter "Antagonist Therapeutics") are administered for therapeutic effect. In another embodiment, Therapeutics which

promote Notch and/or deltex function (hereinafter "Agonist Therapeutics") are administered for therapeutic effect.

Disorders of cell fate, in particular hyperproliferative (*e.g.*, cancer) or hypoproliferative disorders, involving aberrant or undesirable levels of expression or activity or localization of Notch and/or deltex protein can be diagnosed by detecting such levels, as described more fully *infra*.

In a preferred aspect, a Therapeutic of the invention is a protein consisting of at least a fragment (termed herein "adhesive fragment") of deltex that mediates binding to a Notch protein, a second deltex protein, or a fragment of Notch or deltex.

The invention is illustrated by way of examples *infra* which disclose, *inter alia*, the cloning and sequencing of *D. melanogaster deltex*; the construction and recombinant expression of *deltex* chimeric/fusion derivatives, production of anti-deltex antibodies, and the identification of regions of deltex which bind to the ANK repeats of Notch, or which bind to regions of deltex.

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections set forth below.

5.1. ISOLATION OF THE *DELTEX* NUCLEIC ACIDS

The invention relates to the nucleotide sequences of *deltex* nucleic acids. In specific embodiments, *Drosophila deltex* nucleic acids comprise the genomic sequence shown in Figure 3 (SEQ ID NO:1), or the cDNA sequence shown in Figure 12 (SEQ ID NO:3), or the coding regions thereof, or nucleic acids encoding a deltex protein (*e.g.*, having the sequence of SEQ ID NO:4). The invention provides nucleic acids consisting of at least 8 nucleotides (*i.e.*, a hybridizable portion) of a *deltex* sequence; in other embodiments, the nucleic acids consist of at least 25 (continuous) nucleotides, 50 nucleotides, 100 nucleotides, 150 nucleotides, or 200 nucleotides of a *deltex* sequence, or a full-length *deltex* coding sequence. The invention also relates to nucleic acids hybridizable to or complementary to the foregoing sequences. In specific aspects, nucleic acids are provided which comprise a sequence complementary to at least

10, 25, 50, 100, or 200 nucleotides or the entire coding region of a *deltex* gene. In a specific embodiment, a nucleic acid which is hybridizable to a *deltex* nucleic acid (*e.g.*, having sequence SEQ ID NO:3), or to a nucleic acid encoding a deltex derivative, under conditions of low stringency is provided. By way of example and not limitation, procedures using such conditions of low stringency are as follows (see also Shilo and Weinberg, 1981, Proc. Natl. Acad. Sci. USA 78:6789-6792): Filters containing DNA are pretreated for 6 h at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 μg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 μg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20 X 10^6 cpm ^{32}P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 h at 40°C, and then washed for 1.5 h at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 h at 60°C. Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film. Other conditions of low stringency which may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations).

In another specific embodiment, a nucleic acid which is hybridizable to a deltex nucleic acid under conditions of high stringency is provided. By way of example and not limitation, procedures using such conditions of high stringency are as follows: Prehybridization of filters containing DNA is carried out for 8 h to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 μg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65°C in prehybridization mixture containing 100 μg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 37°C for 1 h in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X

SSC at 50°C for 45 min before autoradiography. Other conditions of high stringency which may be used are well known in the art.

Nucleic acids encoding fragments and derivatives of deltex proteins (see Section 5.6), and *deltex* antisense nucleic acids (see Section 5.11) are additionally provided. As is readily apparent, as used herein, a "nucleic acid encoding a fragment or portion of a deltex protein" shall be construed as referring to a nucleic acid encoding only the recited fragment or portion of the deltex protein and not the other contiguous portions of the deltex protein as a continuous sequence.

Specific embodiments for the cloning of a *deltex* gene, *e.g.*, a human *deltex* gene, presented as a particular example but not by way of limitation, follows:

For expression cloning (a technique commonly known in the art), an expression library is obtained or is constructed by methods known in the art. For example, mRNA (*e.g.*, human) is isolated, cDNA is made and ligated into an expression vector (*e.g.*, a bacteriophage derivative) such that it is capable of being expressed by the host cell into which it is then introduced. Various screening assays can then be used to select for the expressed deltex product. In a preferred aspect, anti-deltex antibodies can be used to select the recombinant host cell expressing a cloned *deltex* gene.

In another specific embodiment, PCR is used to amplify the desired *deltex* sequence in a genomic or cDNA library, prior to selection. Oligonucleotide primers representing known deltex sequences can be used as primers in PCR. The synthetic oligonucleotides may be utilized as primers to amplify by PCR sequences from a source (RNA or DNA), preferably a cDNA library, of potential interest. PCR can be carried out, *e.g.*, by use of a Perkin-Elmer Cetus thermal cycler and Taq polymerase (Gene Amp™). The DNA being amplified can include human mRNA or cDNA or genomic DNA. One can choose to synthesize several different degenerate primers, for use in the PCR reactions. It is also possible to vary the stringency of hybridization conditions used in priming the PCR reactions, to allow for greater or lesser degrees of

nucleotide sequence similarity between the known *deltex* nucleotide sequence and the nucleic acid homolog being isolated. For cross species hybridization, low stringency conditions are preferred (see *supra*). For same species hybridization, moderately stringent conditions are preferred (see *supra*). After successful amplification of a segment of a *deltex* gene homolog, that segment may be molecularly cloned and sequenced, and utilized as a probe to isolate a complete cDNA or genomic clone. This, in turn, will permit the determination of the gene's complete nucleotide sequence, the analysis of its expression, and the production of its protein product for functional analysis, as described *infra*. In a preferred aspect, human genes encoding deltex proteins may be identified in this fashion. Alternatively to selection by hybridization, the PCR-amplified DNA can be inserted into an expression vector for expression cloning as described above.

In the event that it is desired to isolate a *deltex* gene by cross-species hybridization (either by direct hybridization to a *deltex* probe representing all or a part of a *deltex* gene of an evolutionarily distant, different species, or by PCR using oligonucleotide primers derived from the sequence of a *deltex* gene of a different, evolutionarily distant species), and no hybridizing *deltex* genes are detected or amplified when hybridization is carried out, the desired *deltex* gene can be isolated by a more gradual method via first isolating a *deltex* gene from a more closely related species, identifying the portions of *deltex* which are conserved cross-species, and then screening with a probe or priming for PCR with a nucleic acid containing the conserved sequence. This method, while more cumbersome, is straightforward and can be readily carried out by routine methods. For example, in a specific aspect directed toward isolating a human *deltex* gene, first a gene from another species of fly (*e.g.*, *Drosophila hidei*, *Drosophila pseudoobscura*, mosquito, or housefly) can be isolated, and the conserved portions of the sequence identified, and used to screen or amplify a human cDNA library. One may also, prior to screening or amplifying the human library, isolate "intermediate" *deltex* genes, *e.g.*, from fish, rat, cow, or other species, to further define regions of sequence conserved across species.

The above-methods are not meant to limit the following general description of methods by which clones of *deltex* may be obtained.

Any eukaryotic cell can potentially serve as the nucleic acid source for the molecular cloning of the *deltex* gene. The DNA may be obtained by standard procedures known in the art from cloned DNA (*e.g.*, a DNA "library"), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired human cell (see, for example Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, 2d. Ed., Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K. Vol. I, II.) Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences. Whatever the source, the gene should be molecularly cloned into a suitable vector for propagation of the gene.

In the molecular cloning of the gene from genomic DNA, DNA fragments are generated, some of which will encode the desired gene. The DNA may be cleaved at specific sites using various restriction enzymes. Alternatively, one may use DNAse in the presence of manganese to fragment the DNA, or the DNA can be physically sheared, as for example, by sonication. The linear DNA fragments can then be separated according to size by standard techniques, including but not limited to, agarose and polyacrylamide gel electrophoresis and column chromatography.

Once the DNA fragments are generated, identification of the specific DNA fragment containing the desired gene may be accomplished in a number of ways. For example, if an amount of a portion of a *deltex* (of any species) gene or its specific RNA, or a fragment thereof *e.g.*, the adhesive domain, is available and can be purified and labeled, the generated DNA fragments may be screened by nucleic acid hybridization to the labeled probe (Benton, W. and Davis, R., 1977, Science 196, 180; Grunstein, M. And Hogness, D., 1975, Proc. Natl. Acad. Sci. U.S.A. 72, 3961). Those DNA fragments with substantial homology to the probe will hybridize. For cross

species hybridization, low stringency conditions are preferred (see *supra*). For same species hybridization, moderately stringent conditions are preferred (see *supra*). It is also possible to identify the appropriate fragment by restriction enzyme digestion(s) and comparison of fragment sizes with those expected according to a known restriction map if such is available. Further selection can be carried out on the basis of the properties of the gene. Alternatively, the presence of the gene may be detected by assays based on the physical, chemical, or immunological properties of its expressed product. For example, cDNA clones, or DNA clones which hybrid-select the proper mRNAs, can be selected which produce a protein that, *e.g.*, has similar or identical electrophoretic migration, isolectric focusing behavior, proteolytic digestion maps, *in vitro* aggregation activity ("adhesiveness") or antigenic properties as known for deltex. If an antibody to deltex is available, the deltex protein may be identified by binding of labeled antibody to the putatively deltex synthesizing clones, in an ELISA (enzyme-linked immunosorbent assay)-type procedure.

The *deltex* gene can also be identified by mRNA selection by nucleic acid hybridization followed by *in vitro* translation. In this procedure, fragments are used to isolate complementary mRNAs by hybridization. Such DNA fragments may represent available, purified *deltex* DNA of another species (*e.g.*, *Drosophila*). Immunoprecipitation analysis or functional assays (*e.g.*, aggregation ability *in vitro*; see examples *infra*) of the *in vitro* translation products of the isolated products of the isolated mRNAs identifies the mRNA and, therefore, the complementary DNA fragments that contain the desired sequences. In addition, specific mRNAs may be selected by adsorption of polysomes isolated from cells to immobilized antibodies specifically directed against deltex protein. A radiolabelled *deltex* cDNA can be synthesized using the selected mRNA (from the adsorbed polysomes) as a template. The radiolabelled mRNA or cDNA may then be used as a probe to identify the *deltex* DNA fragments from among other genomic DNA fragments.

Alternatives to isolating the *deltex* genomic DNA include, but are not limited to, chemically synthesizing the gene sequence itself from a known

sequence or making cDNA to the mRNA which encodes the *deltex* gene. For example, RNA for cDNA cloning of the *deltex* gene can be isolated from cells which express deltex. Other methods are possible and within the scope of the invention.

The identified and isolated gene can then be inserted into an appropriate cloning vector. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as PBR322 or pUC plasmid derivatives. The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and *deltex* gene may be modified by homopolymeric tailing. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

In an alternative method, the desired gene may be identified and isolated after insertion into a suitable cloning vector in a "shot gun" approach. Enrichment for the desired gene, for example, by size fractionization, can be done before insertion into the cloning vector.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated *deltex* gene, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene may be obtained in large quantities by growing transformants,

isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

The *deltex* sequences provided by the instant invention include those nucleotide sequences encoding substantially the same amino acid sequences as found in native deltex protein, and those encoded amino acid sequences with functionally equivalent amino acids, all as described in Section 5.6 *infra* for deltex derivatives.

5.2. EXPRESSION OF *DELTEX* NUCLEIC ACIDS

The nucleic acid coding for a deltex protein or a functionally active fragment or other derivative thereof can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The necessary transcriptional and translational signals can also be supplied by the native *deltex* gene and/or its flanking regions. A variety of host-vector systems may be utilized to express the protein-coding sequence. These include but are not limited to mammalian cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (*e.g.*, baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used. In a specific embodiment, a molecule comprising a portion of a *deltex* gene which encodes a protein that binds to Notch or a molecule comprising the Notch ANK repeats is expressed. In another embodiment, a molecule comprising a portion of a *deltex* gene which encodes a protein that binds to a fragment of a deltex protein is expressed. In other specific embodiments, the human *deltex* gene is expressed, or a sequence encoding a functionally active portion of human deltex. In a specific embodiment, a chimeric protein comprising a Notch-binding domain of a deltex protein is expressed. In other specific embodiments, a full-length *deltex* cDNA is expressed, or a

sequence encoding a functionally active portion of a deltex protein. In yet another embodiment, a fragment of a deltex protein comprising a domain of the protein, or other derivative, or analog of a deltex protein is expressed.

Any of the methods previously described for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a chimeric gene consisting of appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of a nucleic acid sequence encoding a deltex protein or peptide fragment may be regulated by a second nucleic acid sequence so that the deltex protein or peptide is expressed in a host transformed with the recombinant DNA molecule. For example, expression of a deltex protein may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control deltex gene expression include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the β-lactamase (Villa-Kamaroff et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), *tac* (DeBoer et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25), λP_L, or *trc* promoters; see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; plant expression vectors comprising the nopaline synthetase promoter region or the cauliflower mosaic virus 35S RNA promoter (Gardner et al., 1981, Nucl. Acids Res. 9:2871), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella et al., 1984, Nature 310:115-120); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity

and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58; alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94; myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

Expression vectors containing *deltex* gene inserts can be identified by three general approaches: (a) nucleic acid hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a foreign gene inserted in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted *deltex* gene. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (*e.g.*, thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign genes in the

vector. For example, if the *deltex* gene is inserted within the marker gene sequence of the vector, recombinants containing the *deltex* insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the foreign gene product expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the *deltex* gene product in *in vitro* assay systems, *e.g.*, binding to Notch, binding with antibody.

Once a particular recombinant DNA molecule is identified and isolated, several methods known in the art may be used to propagate it. Once a suitable host system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As previously explained, the expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (*e.g.*, lambda), and plasmid and cosmid DNA vectors, to name but a few.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered deltex protein may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, phosphorylation, cleavage) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed.

In other specific embodiments, the deltex protein, fragment, analog, or derivative may be expressed as a fusion, or chimeric protein product (comprising the protein, fragment, analog, or derivative joined via a peptide bond to a heterologous protein sequence (of a different protein)). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in

the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer.

Both cDNA and genomic sequences can be cloned and expressed.

In other embodiments, a *deltex* cDNA sequence may be chromosomally integrated and expressed. Homologous recombination procedures known in the art may be used.

5.3. IDENTIFICATION AND PURIFICATION OF THE *DELTEX* GENE PRODUCTS

In particular aspects, the invention provides amino acid sequences of deltex, preferably human deltex, and fragments and derivatives thereof which comprise an antigenic determinant (*i.e.*, can be recognized by an antibody) or which are functionally active, as well as nucleic acid sequences encoding the foregoing. "Functionally active" material as used herein refers to that material displaying one or more known functional activities associated with the full-length (wild-type) deltex protein product, *e.g.*, binding to Notch or a portion thereof, binding to another deltex molecule or portion thereof, binding to any other deltex ligand, antigenicity (binding to an anti-deltex antibody), Notch intracellular signal transduction, etc.

In specific embodiments, the invention provides fragments of a deltex protein consisting of at least 6 amino acids, 10 amino acids, 50 amino acids, or of at least 75 amino acids. In other embodiments, the proteins comprise or consist essentially of one or more Glu-rich clusters (*e.g.*, amino acids 261-302 and amino acids 488-513 of SEQ ID NO:4); one or more of the SH3 binding domains (*e.g.*, SEQ ID NOS:11 and 12 of Fig. 16), or a portion which binds to Notch (*e.g.*, comprising the first approximately 200 amino acids of deltex), or any combination of the foregoing, of a deltex protein. Fragments, or proteins comprising fragments, lacking some or all of the foregoing regions of deltex are also provided. Nucleic acids encoding the foregoing are provided.

Once a recombinant which expresses a *deltex* gene sequence is identified, the gene product can be analyzed. This is achieved by assays based on

the physical or functional properties of the product, including radioactive labelling of the product followed by analysis by gel electrophoresis, immunoassay, etc. Chemically synthesized proteins, derivatives, and analogs can be similarly analyzed.

Once a deltex protein is identified, it may be isolated and purified by standard methods including chromatography (*e.g.*, ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. The functional properties may be evaluated using any suitable assay (see Section 5.7).

Alternatively, the amino acid sequence of a deltex protein can be deduced from the nucleotide sequence of the chimeric gene contained in the recombinant. Once the amino acid sequence is thus known, the protein can be synthesized by standard chemical methods known in the art (*e.g.*, see Hunkapiller et al., 1984, Nature 310:105-111).

By way of example, the deduced amino acid sequence (SEQ ID NOS:2 or 4 (which are identical to each other) of a *Drosophila* deltex protein is presented in Figure 12.

5.4. STRUCTURE OF THE *DELTEX* GENES AND PROTEINS

The structure of the deltex genes and proteins can be analyzed by various methods known in the art.

5.4.1. GENETIC ANALYSIS

The cloned DNA or cDNA corresponding to the *deltex* gene can be analyzed by methods including but not limited to Southern hybridization (Southern, 1975, J. Mol. Biol. 98:503-517), Northern hybridization (see *e.g.*, Freeman et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:4094-4098, and Section 6.1.3, *infra*), restriction endonuclease mapping (Maniatis, 1982, Molecular Cloning, A Laboratory, Cold Spring Harbor, New York), and DNA sequence analysis (see Section 6.3.1 and Figs. 1-4). Polymerase chain reaction (PCR; U.S. Patent Nos. 4,683,202, 4,683,195 and 4,889,818; Gyllenstein et al., 1988, Proc.

Natl. Acad. Sci. U.S.A. 85:7652-7656; Ochman et al., 1988, Genetics 120:621-623; Loh et al., 1989, Science 243:217-220) followed by Southern hybridization with a *deltex*-specific probe can allow the detection of the *deltex* genes in DNA from various cell types. In one embodiment, Southern hybridization can be used to determine the genetic linkage of *deltex*. Northern hybridization analysis can be used to determine the expression of the *deltex* genes. Various cell types, at various states of development or activity can be tested for *deltex* gene expression. The stringency of the hybridization conditions for both Southern and Northern hybridization can be manipulated to ensure detection of nucleic acids with the desired degree of relatedness to the specific *deltex* probe used.

Restriction endonuclease mapping can be used to roughly determine the genetic structure of the *deltex* gene. Restriction maps derived by restriction endonuclease cleavage can be confirmed by DNA sequence analysis. Alternatively, restriction maps can be deduced, once the nucleotide sequence is known.

DNA sequence analysis can be performed by any techniques known in the art, including but not limited to the method of Maxam and Gilbert (1980, Meth. Enzymol. 65:499-560), the Sanger dideoxy method (Sanger et al., 1977, Proc. Natl. Acad. Sci. U.S.A. 74:5463), the use of T7 DNA polymerase (Tabor and Richardson, U.S. Patent No. 4,795,699; Sequenase, U.S. Biochemical Corp.), or Taq polymerase, or use of an automated DNA sequenator (*e.g.*, Applied Biosystems, Foster City, CA). The cDNA sequence of a *Drosophila melanogaster deltex* gene is shown in Figure 12 (SEQ ID NO:3) and is described in Section 6, *infra*.

5.4.2. PROTEIN ANALYSIS

The amino acid sequence of a deltex protein can be derived by deduction from the DNA sequence, or alternatively, by direct sequencing of the protein, *e.g.*, with an automated amino acid sequencer. The amino acid sequence of a representative deltex protein comprises the sequence substantially as depicted in Figure 12 (SEQ ID NO:4), and detailed in Section 6, *infra*.

The deltex protein sequence can be further characterized by a hydrophilicity analysis (Hopp and Woods, 1981, Proc. Natl. Acad. Sci. U.S.A. 78:3824). A hydrophilicity profile can be used to identify the hydrophobic and hydrophilic regions of a deltex protein and the corresponding regions of the gene sequence which encode such regions.

Secondary, structural analysis (Chou and Fasman, 1974, Biochemistry 13:222) can also be done, to identify regions of a deltex protein that assume specific secondary structures.

Manipulation, translation, and secondary structure prediction, as well as open reading frame prediction and plotting, can also be accomplished using computer software programs available in the art.

Other methods of structural analysis can also be employed. These include but are not limited to X-ray crystallography (Engstom, 1974, Biochem. Exp. Biol. 11:7-13) and computer modeling (Fletterick and Zoller (eds.), 1986, Computer Graphics and Molecular Modeling, in Current Communications in Molecular Biology, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York).

5.5. GENERATION OF ANTIBODIES TO DELTEX PROTEINS AND DERIVATIVES THEREOF

According to the invention, a deltex protein, its fragments or other derivatives, or analogs thereof, may be used as an immunogen to generate antibodies which recognize such an immunogen. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library. In a preferred embodiment, antibodies which specifically bind to deltex proteins are produced. In another embodiment, antibodies to a particular domain of a deltex protein are produced. In a specific embodiment, an antibody is produced which binds to a fragment of deltex which binds to Notch; in another embodiment, an antibody binds to a molecule comprising the first 204 amino-terminal amino acids of deltex. In another embodiment the antibody binds to an amino-terminal fragment of deltex containing not more than the first 200 amino acids of deltex. In yet another

embodiment, an antibody binds to a fragment of deltex which binds to a second deltex molecule.

Various procedures known in the art may be used for the production of polyclonal antibodies to a deltex protein or derivative or analog. In a particular embodiment, rabbit polyclonal antibodies to an epitope of the deltex protein having a sequence depicted in Figure 12 or a subsequence thereof, can be obtained. For the production of antibody, various host animals can be immunized by injection with a native deltex protein, or a synthetic version, or derivative (*e.g.*, fragment) thereof, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

In a preferred embodiment, polyclonal or monoclonal antibodies are produced by use of a hydrophilic portion of a deltex peptide (*e.g.*, identified by the procedure of Hopp and Woods (1981, Proc. Natl. Acad. Sci. U.S.A. 78:3824)).

For preparation of monoclonal antibodies directed toward a deltex protein sequence or analog thereof, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) can be used. In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals (PCT Publication No. WO 89/12690 dated December 28, 1989). According to the invention, human

antibodies may be used and can be obtained by using human hybridomas (Cote et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:2026-2030) or by transforming human B cells with EBV virus *in vitro* (Cole et al., 1985, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, pp. 77-96), or by other methods known in the art. In fact, according to the invention, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851-6855; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing the genes from a mouse antibody molecule specific for a deltex protein together with genes from a human antibody molecule of appropriate biological activity can be used; such antibodies are within the scope of this invention.

According to the invention, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce deltex protein-specific single chain antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries (Huse et al., 1989, Science 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for deltex proteins, derivatives, or analogs.

Antibody fragments and other derivatives which contain the idiotype (binding domain) of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, *e.g.* ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies which recognize a specific domain of a deltex protein, one may assay generated hybridomas for a product which binds to a deltex fragment containing such domain. For selection of an antibody specific to human deltex protein(s), one can select on the basis of

positive binding to a human deltex protein and a lack of binding to *Drosophila* deltex protein.

In a specific embodiment, antibodies specific to a phosphorylated epitope of deltex are produced.

The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the protein sequences of the invention *e.g.*, for imaging these proteins, measuring levels thereof in appropriate physiological samples, etc. Antibodies to deltex (since it normally colocalizes with Notch) can be used to determine the intracellular distribution of Notch and/or deltex, in diagnostic methods such as described *infra*. The antibodies also have use in immunoassays. In another embodiment of the invention (see *infra*), anti-deltex antibodies and fragments thereof containing the binding domain are Therapeutics.

5.6. DELTEX PROTEINS, DERIVATIVES AND ANALOGS

The invention further provides deltex proteins, and derivatives (including but not limited to fragments) and analogs of deltex proteins. Nucleic acids encoding deltex protein derivatives and protein analogs are also provided. In one embodiment, the deltex proteins are encoded by the deltex nucleic acids described in Section 5.1 *supra*. In particular aspects, the proteins, derivatives, or analogs are of fly, frog, mouse, rat, pig, cow, dog, monkey, or human deltex proteins.

The production and use of derivatives and analogs related to deltex are within the scope of the present invention. In a specific embodiment, the derivative or analog is functionally active, *i.e.*, capable of exhibiting one or more functional activities associated with a full-length, wild-type deltex protein.

In particular, deltex derivatives can be made by altering deltex sequences by substitutions, additions or deletions that provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as a *deltex* gene may be used in the practice of the present invention. These

include but are not limited to nucleotide sequences comprising all or portions of *deltex* genes which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change. Likewise, the deltex derivatives of the invention include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of a deltex protein including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a silent change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

In a specific embodiment of the invention, proteins consisting of or comprising a fragment of a deltex protein consisting of at least 10 (continuous) amino acids of the deltex protein is provided. In other embodiments, the fragment consists of at least 20 or 50 amino acids of the deltex protein. In specific embodiments, such fragments are not larger than 35, 100 or 200 amino acids. Derivatives or analogs of deltex include but are not limited to those peptides which are substantially homologous to deltex or fragments thereof (*e.g.*, at least 30% identity over an amino acid sequence of identical size) or whose encoding nucleic acid is capable of hybridizing to a coding *deltex* sequence.

The deltex protein derivatives and analogs of the invention can be produced by various methods known in the art. The manipulations which result in their production can occur at the gene or protein level. For example, the cloned *deltex* gene sequence can be modified by any of numerous strategies

known in the art (Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The sequence can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated *in vitro*. In the production of the gene encoding a derivative or analog of a deltex protein, care should be taken to ensure that the modified gene remains within the same translational reading frame as the *deltex* gene, uninterrupted by translational stop signals, in the gene region where the desired deltex activity is encoded.

Additionally, the deltex-encoding nucleic acid sequence can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy preexisting ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art can be used, including but not limited to, *in vitro* site-directed mutagenesis (Hutchinson et al., 1978, J. Biol. Chem 253:6551), use of TAB® linkers (Pharmacia), etc.

Manipulations of the deltex sequence may also be made at the protein level. Included within the scope of the invention are deltex protein fragments or other derivatives or analogs which are differentially modified during or after translation, *e.g.*, by acetylation, phosphorylation, carboxylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$, acetylation, formylation, oxidation, reduction, etc.

In a preferred aspect, phosphorylation or, alternatively, dephosphorylation is carried out, which can be to various extents, on the purified deltex protein or derivative thereof. The phosphorylation state of the molecule may be important to its role in intracellular signal transduction of Notch function. Phosphorylation can be carried out by reaction with an appropriate kinase (*e.g.*,

possibly cdc2 or CK II). Dephosphorylation can be carried out by reaction with an appropriate phosphatase.

In addition, analogs and derivatives of deltex proteins can be chemically synthesized. For example, a peptide corresponding to a portion of a deltex protein which comprises the desired domain, or which mediates the desired activity *in vitro*, can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the deltex protein sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, α-amino isobutyric acid, 4-aminobutyric acid, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, and Nα-methyl amino acids.

In a specific embodiment, the deltex derivative is a chimeric, or fusion, protein comprising a deltex protein or fragment thereof (preferably consisting of at least a domain or motif of the deltex protein, or at least 10 amino acids of the deltex protein) joined at its amino or carboxy-terminus via a peptide bond to an amino acid sequence of a different protein. In one embodiment, such a chimeric protein is produced by recombinant expression of a nucleic acid encoding the protein (comprising a deltex-coding sequence joined in-frame to a coding sequence for a different protein). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer. A specific embodiment relates to a chimeric protein comprising a fragment of a deltex protein which comprises a domain or motif of the deltex protein, *e.g.*, a Glu-rich cluster, portion which binds to a Notch protein or to a second deltex protein, an SH3 binding domain, etc. In a particular embodiment, a chimeric nucleic acid can be constructed, encoding a fusion protein consisting of a deltex Notch-binding fragment joined to

a non-deltex protein. As another example, and not by way of limitation, a recombinant molecule can be constructed according to the invention, comprising coding portions of both a *deltex* gene and another gene which is a member of the "Notch group." Another specific embodiment relates to a chimeric protein comprising a fragment of a deltex protein of at least six amino acids. Particular examples of the construction and expression of fusion proteins comprising deltex or various deltex fragments, are described in Sections 7 and 8 hereof.

Other specific embodiments of derivatives and analogs are described in the subsection below and examples sections *infra*.

5.6.1. DERIVATIVES OF DELTEX CONTAINING ONE OR MORE DOMAINS OF THE PROTEIN

In a specific embodiment, the invention provides deltex derivatives and analogs, in particular deltex fragments and derivatives of such fragments, that comprise or consist of one or more domains of the deltex protein, including but not limited to a Glu-rich cluster, a region which binds to a Notch protein (or a molecule comprising the ANK repeats thereof), a region which binds to a second deltex protein or portion thereof, or an SH3-binding domain. In specific embodiments, the deltex derivative may lack all or a portion of one or more of the foregoing domains.

In specific embodiments directed to the domains of the *D. melanogaster* deltex protein, the aforesaid domains consist of approximately the following amino-acid sequences:

Glu-rich clusters:	amino acids 261-302 of Figure 12 (part of SEQ ID NO:4);
	amino acids 488-513 of Figure 12 (part of SEQ ID NO:4);
SH3 binding domain:	SEQ ID NO:10 of Figure 16
	SEQ ID NO:11 of Figure 16
Notch-binding fragment:	Fragment D (SEQ ID NO:8) of Figure 14

Deltex-binding fragments:	Fragment A (SEQ ID NO:5) of Figure 14
	Fragment B (SEQ ID NO:6) of Figure 14
	Fragment C (SEQ ID NO:7) of Figure 14

Other binding fragments, *e.g.*, smaller than those set forth above, can be identified by routine methods, *e.g.*, by construction of nucleic acids encoding such fragments and assays for binding (*e.g.* via the interaction trap method, S2 cell expression assay) such as described in Sections 7 and 8 *infra*.

In a specific embodiment, relating to a deltex protein of a species other than *D. melanogaster*, preferably human, fragments comprising specific domains of deltex are those comprising domains in the respective deltex protein most homologous to the specific domain of the *Drosophila melanogaster* deltex protein.

In specific embodiments, deltex fragments which homotypically bind to an identical deltex fragment (*e.g.* fragment A (SEQ ID NO:5), fragment B (SEQ ID NO:6), and fragment C (SEQ ID NO:7) shown in Fig. 14) or to a distinct deltex fragment (*e.g.*, fragment B (SEQ ID NO:6) and fragment C (SEQ ID NO:7) shown in Fig. 14) are provided. Examples of such fragments and assays for their selection are described in Section 8 *infra*.

Also provided are inhibitors (*e.g.*, peptide inhibitors) of the foregoing protein interactions with Notch or with a second deltex protein.

The ability to bind to a Notch protein or a deltex protein (or derivative thereof) can be demonstrated by *in vitro* assays such as described in Sections 7 and 8 *infra*, *e.g.*, by S2 cell expression assay (Section 7.3), interaction trap technique (Sections 7.4, 8), etc.

The nucleic acid sequences encoding Notch or deltex proteins or fragments thereof, for use in such assays, can be isolated from human, porcine, bovine, feline, avian, equine, canine, or insect, as well as primate sources and any other species in which homologs of known genes can be identified. For

example, the Notch protein or portion thereof comprising the ANK repeats which can be expressed and assayed for binding to deltex or a deltex derivative can be or be derived from any of the Notch homologs shown in Figure 17 (human hN, human TAN-1, *Xenopus*, and *Drosophila*; SEQ ID NOS:16-19, respectively).

Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as the aforesaid domains may be used in the practice of the present invention. These include but are not limited to nucleotide sequences comprising all or portions of the *deltex* genes which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change. Likewise, the deltex proteins, fragments or derivatives thereof, of the invention include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of the domains including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence ("conservative" changes).

The derivatives, analogs, and peptides of the invention can be produced by various methods known in the art. The manipulations which result in their production can occur at the gene or protein level.

Additionally, the nucleic acid sequence can be mutated *in vitro* or *in vivo*; and manipulations of the sequence may also be made at the protein level.

In addition, analogs and peptides can be chemically synthesized.

5.7. *IN VITRO* ASSAYS OF DELTEX PROTEINS, DERIVATIVES AND ANALOGS

The functional activity of deltex proteins, derivatives and analogs, can be assayed *in vitro* by various methods.

For example, in one embodiment, where one is assaying for the ability to bind or compete with wild-type deltex for binding to anti-deltex antibody, various immunoassays known in the art can be used, including but not limited to competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions,

immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labelled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where one is assaying for the ability to mediate binding to Notch or to a second deltex protein or portions thereof, one can carry out assays such as described *infra* in Section 7 or 8.

Other methods will be known to the skilled artisan and are within the scope of the invention.

5.8. THERAPEUTIC USES

The invention provides for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: deltex proteins and analogs and derivatives (including fragments) thereof (*e.g.*, as described hereinabove); antibodies thereto (as described hereinabove); nucleic acids encoding the deltex proteins, analogs, or derivatives (*e.g.*, as described hereinabove); and *deltex* antisense nucleic acids. As stated *supra*, the Antagonist Therapeutics of the invention are those Therapeutics which antagonize, or inhibit, a deltex function and/or Notch function (since the data (see Sections 6-9) indicates that deltex functions in intracellular signal transduction from Notch). Such Antagonist Therapeutics are most preferably identified by use of known convenient *in vitro* assays, *e.g.*, based on their ability to inhibit binding of deltex to another protein (*e.g.*, a Notch protein), or inhibit any known Notch or deltex function as preferably assayed *in vitro* or in cell culture, although genetic assays

(*e.g.*, in *Drosophila*) may also be employed. In a preferred embodiment, the Antagonist Therapeutic is a protein or derivative thereof comprising a functionally active fragment such as a fragment of deltex which mediates binding to Notch, or an antibody thereto. In other specific embodiments, such an Antagonist Therapeutic is a nucleic acid capable of expressing a molecule comprising a fragment of deltex which binds to Notch, or a *deltex* antisense nucleic acid (see Section 5.11 herein). It should be noted that preferably, suitable *in vitro* or *in vivo* assays, as described *infra*, should be utilized to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue, since the developmental history of the tissue may determine whether an Antagonist or Agonist Therapeutic is desired.

In another embodiment of the invention, a nucleic acid containing a portion of a *deltex* gene is used, as an Antagonist Therapeutic, to promote *deltex* inactivation by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438).

The Agonist Therapeutics of the invention, as described *supra*, promote deltex function. Such Agonist Therapeutics include but are not limited to proteins and derivatives comprising the portions of Notch that mediate binding to deltex, *i.e.*, the ANK repeats, and nucleic acids encoding the foregoing (which can be administered to express their encoded products *in vivo*).

Further descriptions and sources of Therapeutics of the inventions are found in Sections 5.1 through 5.7 herein.

Molecules which retain, or alternatively inhibit, a desired deltex property, *e.g.*, binding to Notch, binding to an intracellular ligand, can be used therapeutically as inducers, or inhibitors, respectively, of such property and its physiological correlates. In a specific embodiment, a peptide (*e.g.*, in the range of 6-50 or 15-25 amino acids; and particularly of about 10, 15, 20 or 25 amino acids) containing the sequence of a portion of deltex which binds to Notch is used to antagonize Notch function. In a specific embodiment, such an Antagonist Therapeutic is used to treat or prevent human or other malignancies associated

with increased Notch expression (*e.g.*, cervical cancer, colon cancer, breast cancer, squamous adenocarcimas (see *infra*)). Derivatives or analogs of deltex can be tested for the desired activity by procedures known in the art, including but not limited to the assays described in the examples *infra*. For example, molecules comprising deltex fragments which bind to Notch ANK repeats and which are smaller than deltex fragment D (see Figure 14 and Section 8), can be obtained and selected by expressing deletion mutants of fragment D (or of a nucleotide sequence of another species encoding the amino-terminal ~204 amino acids of deltex) and assaying for binding of the expressed product to Notch by any of the several methods (*e.g.*, interaction trap system) described in the Examples Sections *infra*. In one specific embodiment, peptide libraries can be screened to select a peptide with the desired activity; such screening can be carried out by assaying, *e.g.*, for binding to Notch or a molecule containing the Notch ANK repeats.

The Agonist and Antagonist Therapeutics of the invention have therapeutic utility for disorders of cell fate. The Agonist Therapeutics are administered therapeutically (including prophylactically): (1) in diseases or disorders involving an absence or decreased (relative to normal, or desired) levels of Notch or deltex function, for example, in patients where Notch or deltex protein is lacking, genetically defective, biologically inactive or underactive, or underexpressed; and (2) in diseases or disorders wherein *in vitro* (or *in vivo*) assays (see *infra*) indicate the utility of deltex agonist administration. The absence or decreased levels in Notch or deltex function can be readily detected, *e.g.*, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for protein levels, structure and/or activity of the expressed Notch or deltex protein. Many methods standard in the art can be thus employed, including but not limited to immunoassays to detect and/or visualize Notch or deltex protein (*e.g.*, Western blot, immunoprecipitation followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect Notch or deltex expression by detecting

and/or visualizing respectively Notch or deltex mRNA (*e.g.*, Northern assays, dot blots, *in situ* hybridization, etc.)

In vitro assays which can be used to determine whether administration of a specific Agonist Therapeutic or Antagonist Therapeutic is indicated, include *in vitro* cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a Therapeutic, and the effect of such Therapeutic upon the tissue sample is observed. In one embodiment, where the patient has a malignancy, a sample of cells from such malignancy is plated out or grown in culture, and the cells are then exposed to a Therapeutic. A Therapeutic which inhibits survival or growth of the malignant cells (*e.g.*, by promoting terminal differentiation) is selected for therapeutic use *in vivo*. Many assays standard in the art can be used to assess such survival and/or growth; for example, cell proliferation can be assayed by measuring ^{3}H-thymidine incorporation, by direct cell count, by detecting changes in transcriptional activity of known genes such as proto-oncogenes (*e.g.*, *fos*, *myc*) or cell cycle markers; cell viability can be assessed by trypan blue staining, differentiation can be assessed visually based on changes in morphology, etc. In a specific aspect, the malignant cell cultures are separately exposed to (1) an Agonist Therapeutic, and (2) an Antagonist Therapeutic; the result of the assay can indicate which type of Therapeutic has therapeutic efficacy.

In another embodiment, a Therapeutic is indicated for use which exhibits the desired effect, inhibition or promotion of cell growth, upon a patient cell sample from tissue having or suspected of having a hyper- or hypoproliferative disorder, respectively. Such hyper- or hypoproliferative disorders include but are not limited to those described in Sections 5.8.1 through 5.8.3 *infra*.

In another specific embodiment, a Therapeutic is indicated for use in treating nerve injury or a nervous system degenerative disorder (see Section 5.8.2) which exhibits *in vitro* promotion of nerve regeneration/neurite extension from nerve cells of the affected patient type.

In addition, administration of an Antagonist Therapeutic of the invention is also indicated in diseases or disorders determined or known to involve a Notch or deltex dominant activated phenotype ("gain of function" mutations.) Administration of an Agonist Therapeutic is indicated in diseases or disorders determined or known to involve a Notch or deltex dominant negative phenotype ("loss of function" mutations). The functions of various structural domains of the Notch protein have been investigated *in vivo*, by ectopically expressing a series of *Drosophila Notch* deletion mutants under the hsp70 heat-shock promoter, as well as eye-specific promoters (see Rebay et al., 1993, Cell 74:319-329). Two classes of dominant phenotypes were observed, one suggestive of *Notch* loss-of function mutations and the other of *Notch* gain-of-function mutations. Dominant "activated" phenotypes resulted from overexpression of a protein lacking most extracellular sequences, while dominant "negative" phenotypes resulted from overexpression of a protein lacking most intracellular sequences. The results indicated that Notch functions as a receptor whose extracellular domain mediates ligand-binding, resulting in the transmission of developmental signals by the cytoplasmic domain. The phenotypes observed also suggested that the ANK repeat region within the intracellular domain plays an essential role in Notch mediated signal transduction events (intracellular function). We have shown that deltex binds to the Notch ANK repeat region (see Sections 6-8).

In various specific embodiments, *in vitro* assays can be carried out with representative cells of cell types involved in a patient's disorder, to determine if a Therapeutic has a desired effect upon such cell types.

In another embodiment, cells of a patient tissue sample suspected of being pre-neoplastic are similarly plated out or grown *in vitro*, and exposed to a Therapeutic. The Therapeutic which results in a cell phenotype that is more normal (*i.e.*, less representative of a pre-neoplastic state, neoplastic state, malignant state, or transformed phenotype) is selected for therapeutic use. Many assays standard in the art can be used to assess whether a pre-neoplastic state, neoplastic state, or a transformed or malignant phenotype, is present. For

example, characteristics associated with a transformed phenotype (a set of *in vitro* characteristics associated with a tumorigenic ability *in vivo*) include a more rounded cell morphology, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, release of proteases such as plasminogen activator, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250,000 dalton surface protein, etc. (see Luria et al., 1978, *General Virology*, 3d Ed., John Wiley & Sons, New York pp. 436-446).

In other specific embodiments, the *in vitro* assays described *supra* can be carried out using a cell line, rather than a cell sample derived from the specific patient to be treated, in which the cell line is derived from or displays characteristic(s) associated with the malignant, neoplastic or pre-neoplastic disorder desired to be treated or prevented, or is derived from the neural or other cell type upon which an effect is desired, according to the present invention.

The Antagonist Therapeutics are administered therapeutically (including prophylactically): (1) in diseases or disorders involving increased (relative to normal, or desired) levels of Notch or deltex function, for example, where the Notch or deltex protein is overexpressed or overactive; and (2) in diseases or disorders wherein *in vitro* (or *in vivo*) assays indicate the utility of deltex antagonist administration. The increased levels of Notch or deltex function can be readily detected by methods such as those described above, by quantifying protein and/or RNA. *In vitro* assays with cells of patient tissue sample or the appropriate cell line or cell type, to determine therapeutic utility, can be carried out as described above.

5.8.1. MALIGNANCIES

Malignant and pre-neoplastic conditions which can be tested as described *supra* for efficacy of intervention with Antagonist or Agonist Therapeutics, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to those described below in Sections 5.8.1 and 5.9.1.

Malignancies and related disorders, cells of which type can be tested *in vitro* (and/or *in vivo*), and upon observing the appropriate assay result, treated according to the present invention, include but are not limited to those listed in Table 1 (for a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia):

TABLE 1
MALIGNANCIES AND RELATED DISORDERS

- Leukemia
  - acute leukemia
    - acute lymphocytic leukemia
    - acute myelocytic leukemia
      - myeloblastic
      - promyelocytic
      - myelomonocytic
      - monocytic
      - erythroleukemia
  - chronic leukemia
    - chronic myelocytic (granulocytic) leukemia
    - chronic lymphocytic leukemia
- Polycythemia vera
- Lymphoma
  - Hodgkin's disease
  - non-Hodgkin's disease
- Multiple myeloma
- Waldenström's macroglobulinemia
- Heavy chain disease
- Solid tumors
  - sarcomas and carcinomas
    - fibrosarcoma
    - myxosarcoma
    - liposarcoma
    - chondrosarcoma
    - osteogenic sarcoma
    - chordoma
    - angiosarcoma
    - endotheliosarcoma
    - lymphangiosarcoma
    - lymphangioendotheliosarcoma
    - synovioma
    - mesothelioma

Ewing's tumor
leiomyosarcoma
rhabdomyosarcoma
colon carcinoma
pancreatic cancer
breast cancer
ovarian cancer
prostate cancer
squamous cell carcinoma
basal cell carcinoma
adenocarcinoma
sweat gland carcinoma
sebaceous gland carcinoma
papillary carcinoma
papillary adenocarcinomas
cystadenocarcinoma
medullary carcinoma
bronchogenic carcinoma
renal cell carcinoma
hepatoma
bile duct carcinoma
choriocarcinoma
seminoma
embryonal carcinoma
Wilms' tumor
cervical cancer
testicular tumor
lung carcinoma
small cell lung carcinoma
bladder carcinoma
epithelial carcinoma
glioma
astrocytoma
medulloblastoma
craniopharyngioma
ependymoma
pinealoma
hemangioblastoma
acoustic neuroma
oligodendroglioma
menangioma
melanoma
neuroblastoma
retinoblastoma

In specific embodiments, malignancy or dysproliferative changes (such as metaplasias and dysplasias) are treated or prevented in epithelial tissues such as those in the cervix, esophagus, and lung.

Malignancies of the colon and cervix exhibit increased expression of human Notch relative to such non-malignant tissue (see copending U.S. Application Serial No. 08/083,590 filed June 25, 1993, incorporated by reference herein in its entirety; PCT application no. PCT/US93/09338 filed September 30, 1993, incorporated by reference herein in its entirety). Thus, in specific embodiments, malignancies of the colon or cervix are treated or prevented by administering an effective amount of an Antagonist Therapeutic of the invention. The presence of increased Notch expression in colon, and cervical cancer suggests that many more cancerous and hyperproliferative conditions exhibit upregulated Notch. Thus, in specific embodiments, various cancers, *e.g.*, breast cancer, squamous adenocarcinoma, seminoma, melanoma, and lung cancer, as well as other hyperproliferative disorders, can be treated or prevented by administration of an Antagonist Therapeutic.

5.8.2. NERVOUS SYSTEM DISORDERS

Nervous system disorders, involving cell types which can be tested as described *supra* for efficacy of intervention with Antagonist or Agonist Therapeutics, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to nervous system injuries, and diseases or disorders which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian patients) according to the invention include but are not limited to the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems:

(i) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries;

(ii) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia;

(iii) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue;

(iv) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis;

(v) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis;

(vi) lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration;

(vii) neurological lesions associated with systemic diseases including but not limited to diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis;

(viii) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and

(ix) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including but not limited to multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

Therapeutics which are useful according to the invention for treatment of a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons (see also Section 5.8). For example, and not by way of limitation, Therapeutics which elicit any of the following effects may be useful according to the invention:

(i) increased survival time of neurons in culture;

(ii) increased sprouting of neurons in culture or *in vivo*;

(iii) increased production of a neuron-associated molecule in culture or *in vivo*, *e.g.*, choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or

(iv) decreased symptoms of neuron dysfunction *in vivo*.

Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may be measured by the method set forth in Arakawa et al. (1990, J. Neurosci. 10:3507-3515); increased sprouting of neurons may be detected by methods set forth in Pestronk et al. (1980, Exp. Neurol. 70:65-82) or Brown et al. (1981, Ann. Rev. Neurosci. 4:17-42); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, *e.g.*, weakness, motor neuron conduction velocity, or functional disability.

In a specific embodiments, motor neuron disorders that may be treated according to the invention include but are not limited to disorders such as

infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and including but not limited to progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

5.8.3. TISSUE REPAIR AND REGENERATION

In another embodiment of the invention, a Therapeutic of the invention is used for promotion of tissue regeneration and repair, including but not limited to treatment of benign dysproliferative disorders. Specific embodiments are directed to treatment of cirrhosis of the liver (a condition in which scarring has overtaken normal liver regeneration processes), treatment of keloid (hypertrophic scar) formation (disfiguring of the skin in which the scarring process interferes with normal renewal), psoriasis (a common skin condition characterized by excessive proliferation of the skin and delay in proper cell fate determination), and baldness (a condition in which terminally differentiated hair follicles (a tissue rich in Notch) fail to function properly).

5.9. PROPHYLACTIC USES

5.9.1. MALIGNANCIES

The Therapeutics of the invention can be administered to prevent progression to a neoplastic or malignant state, including but not limited to those disorders listed in Table 1. Such administration is indicated where the Therapeutic is shown in assays, as described *supra*, to have utility for treatment or prevention of such disorder. Such prophylactic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal growth

conditions, see Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-79.) Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. As but one example, endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

Alternatively or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed phenotype, or of a malignant phenotype, displayed *in vivo* or displayed *in vitro* by a cell sample from a patient, can indicate the desirability of prophylactic/therapeutic administration of a Therapeutic of the invention. As mentioned *supra*, such characteristics of a transformed-phenotype include morphology changes, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, protease release, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250,000 dalton cell surface protein, etc. (see also *id.*, at pp. 84-90 for characteristics associated with a transformed or malignant phenotype).

In a specific embodiment, leukoplakia, a benign-appearing hyperplastic or dysplastic lesion of the epithelium, or Bowen's disease, a carcinoma *in situ*, are pre-neoplastic lesions indicative of the desirability of prophylactic intervention.

In another embodiment, fibrocystic disease (cystic hyperplasia, mammary dysplasia, particularly adenosis (benign epithelial hyperplasia)) is indicative of the desirability of prophylactic intervention.

In other embodiments, a patient which exhibits one or more of the following predisposing factors for malignancy is treated by administration of an effective amount of a Therapeutic: a chromosomal translocation associated with a malignancy (*e.g.*, the Philadelphia chromosome for chronic myelogenous leukemia, t(14;18) for follicular lymphoma, etc.), familial polyposis or Gardner's syndrome (possible forerunners of colon cancer), benign monoclonal gammopathy (a possible forerunner of multiple myeloma), and a first degree kinship with persons having a cancer or precancerous disease showing a Mendelian (genetic) inheritance pattern (*e.g.*, familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, medullary thyroid carcinoma with amyloid production and pheochromocytoma, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia, and Bloom's syndrome; *see* Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 112-113) etc.)

In another specific embodiment, an Antagonist Therapeutic of the invention is administered to a human patient to prevent progression to breast, colon, or cervical cancer.

5.9.2. OTHER DISORDERS

In other embodiments, a Therapeutic of the invention can be administered to prevent a nervous system disorder described in Section 5.8.2, or other disorder (*e.g.*, liver cirrhosis, psoriasis, keloids, baldness) described in Section 5.8.3.

5.10. DEMONSTRATION OF THERAPEUTIC OR PROPHYLACTIC UTILITY

The Therapeutics of the invention can be tested *in vivo* for the desired therapeutic or prophylactic activity. For example, such compounds can be tested in suitable animal model systems prior to testing in humans, including but not limited to rats, mice, chicken, cows, monkeys, rabbits, etc. For *in vivo* testing, prior to administration to humans, any animal model system known in the art may be used.

5.11. ANTISENSE REGULATION OF *DELTEX* EXPRESSION

The present invention provides the therapeutic or prophylactic use of nucleic acids of at least six nucleotides that are antisense to a gene or cDNA encoding deltex or a portion thereof. "Antisense" as used herein refers to a nucleic acid capable of hybridizing to a portion of a *deltex* RNA (preferably mRNA) by virtue of some sequence complementarity. Such antisense nucleic acids have utility as Antagonist Therapeutics of the invention, and can be used in the treatment or prevention of disorders as described *supra* in Section 5.8 and its subsections.

The antisense nucleic acids of the invention can be oligonucleotides that are double-stranded or single-stranded, RNA or DNA or a modification or derivative thereof, which can be directly administered to a cell, or which can be produced intracellularly by transcription of exogenous, introduced sequences.

In a specific embodiment, the *deltex* antisense nucleic acids provided by the instant invention can be used for the treatment of tumors or other disorders, the cells of which tumor type or disorder can be demonstrated (*in vitro* or *in vivo*) to express a *deltex* gene or a *Notch* gene. Such demonstration can be by detection of RNA or of protein.

The invention further provides pharmaceutical compositions comprising an effective amount of the *deltex* antisense nucleic acids of the invention in a pharmaceutically acceptable carrier, as described *infra* in Section 5.12. Methods for treatment and prevention of disorders (such as those described

in Sections 5.8 and 5.9) comprising administering the pharmaceutical compositions of the invention are also provided.

In another embodiment, the invention is directed to methods for inhibiting the expression of a *deltex* nucleic acid sequence in a prokaryotic or eukaryotic cell comprising providing the cell with an effective amount of a composition comprising an antisense *deltex* nucleic acid of the invention.

deltex antisense nucleic acids and their uses are described in detail below.

5.11.1. *DELTEX* ANTISENSE NUCLEIC ACIDS

The *deltex* antisense nucleic acids are of at least six nucleotides and are preferably oligonucleotides (ranging from 6 to about 50 oligonucleotides). In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appending groups such as peptides, or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO 88/09810, published December 15, 1988) or blood-brain barrier (see, *e.g.*, PCT Publication No. WO 89/10134, published April 25, 1988), hybridization-triggered cleavage agents (see, *e.g.*, Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents (see, *e.g.*, Zon, 1988, Pharm. Res. 5:539-549).

In a preferred aspect of the invention, a *deltex* antisense oligonucleotide is provided, preferably of single-stranded DNA. In a most preferred aspect, such an oligonucleotide comprises a sequence antisense to the sequence encoding an SH3 binding domain or a Notch-binding domain of deltex, most preferably, of human deltex. The oligonucleotide may be modified at any position on its structure with substituents generally known in the art.

The deltex antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

In another embodiment, the oligonucleotide comprises at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the oligonucleotide is an α-anomeric oligonucleotide. An α-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641).

The oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

Oligonucleotides of the invention may be synthesized by standard methods known in the art, *e.g.* by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

In a specific embodiment, the *deltex* antisense oligonucleotide comprises catalytic RNA, or a ribozyme (see, *e.g.*, PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al., 1990, Science 247:1222-1225). In another embodiment, the oligonucleotide is a 2'-0-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

In an alternative embodiment, the *deltex* antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector can be introduced *in vivo* such that it is taken up by a cell, within which cell the vector or a portion thereof is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the *deltex* antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the deltex antisense RNA can be by any promoter known in the art to act in mammalian, preferably human, cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc.

Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a *deltex* gene, preferably a human *deltex* gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded deltex antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with a *deltex* RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

5.11.2. THERAPEUTIC UTILITY OF *DELTEX* ANTISENSE NUCLEIC ACIDS

The *deltex* antisense nucleic acids can be used to treat (or prevent) malignancies or other disorders, of a cell type which has been shown to express *deltex* or *Notch*. In specific embodiments, the malignancy is cervical, breast, or colon cancer, or squamous adenocarcinoma. Malignant, neoplastic, and pre-neoplastic cells which can be tested for such expression include but are not limited to those described *supra* in Sections 5.8.1 and 5.9.1. In a preferred embodiment, a single-stranded DNA antisense deltex oligonucleotide is used.

Malignant (particularly, tumor) cell types which express *deltex* or *Notch* RNA can be identified by various methods known in the art. Such methods include but are not limited to hybridization with a *deltex* or *Notch*-specific nucleic acid (*e.g.* by Northern hybridization, dot blot hybridization, *in situ* hybridization), observing the ability of RNA from the cell type to be translated *in vitro* into Notch or Deltex, immunoassay, etc. In a preferred aspect,

primary tumor tissue from a patient can be assayed for Notch or deltex expression prior to treatment, *e.g.*, by immunocytochemistry or *in situ* hybridization.

Pharmaceutical compositions of the invention (see Section 5.12), comprising an effective amount of a *deltex* antisense nucleic acid in a pharmaceutically acceptable carrier, can be administered to a patient having a malignancy which is of a type that expresses *Notch* or *deltex* RNA or protein.

The amount of *deltex* antisense nucleic acid which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. Where possible, it is desirable to determine the antisense cytotoxicity of the tumor type to be treated *in vitro*, and then in useful animal model systems prior to testing and use in humans.

In a specific embodiment, pharmaceutical compositions comprising *deltex* antisense nucleic acids are administered via liposomes, microparticles, or microcapsules. In various embodiments of the invention, it may be useful to use such compositions to achieve sustained release of the *deltex* antisense nucleic acids. In a specific embodiment, it may be desirable to utilize liposomes targeted via antibodies to specific identifiable tumor antigens (Leonetti et al., 1990, Proc. Natl. Acad. Sci. U.S.A. 87:2448-2451; Renneisen et al., 1990, J. Biol. Chem. 265:16337-16342).

5.12. THERAPEUTIC/PROPHYLACTIC ADMINISTRATION AND COMPOSITIONS

The invention provides methods of treatment (and prophylaxis) by administration to a subject of an effective amount of a Therapeutic of the invention. In a preferred aspect, the Therapeutic is substantially purified. The subject is preferably an animal, including but not limited to animals such as cows, pigs, chickens, etc., and is preferably a mammal, and most preferably human.

Various delivery systems are known and can be used to administer a Therapeutic of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, expression by recombinant cells, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a

Therapeutic nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, *e.g.*, in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

In another embodiment, the Therapeutic can be delivered in a vesicle, in particular a liposome (*see* Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; *see* generally *ibid.*)

In yet another embodiment, the Therapeutic can be delivered in a controlled release system. In one embodiment, a pump may be used (*see* Langer,

supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (*see* Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); *see also* Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, *i.e.*, the brain, thus requiring only a fraction of the systemic dose (*see*, *e.g.*, Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

In a specific embodiment, administration of a Therapeutic into a Notch-expressing cell is accomplished by linkage of the Therapeutic to a Delta (or other toporythmic) protein or portion thereof capable of mediating binding to Notch. Contact of a Notch-expressing cell with the linked Therapeutic results in binding of the linked Therapeutic via its Delta portion to Notch on the surface of the cell, followed by uptake of the linked Therapeutic into the Notch-expressing cell.

In a specific embodiment, the Therapeutic is delivered intracellularly (*e.g.*, by expression from a nucleic acid vector, or by linkage to a Delta protein capable of binding to Notch followed by binding and internalization, or by receptor-mediated or diffusion mechanisms).

In a specific embodiment where the Therapeutic is a nucleic acid encoding a protein Therapeutic, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun;

Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see *e.g.*, Joliot et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868), etc. Alternatively, a nucleic acid Therapeutic can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

In specific embodiments directed to treatment or prevention of particular disorders, preferably the following forms of administration are used:

Disorder	Preferred Forms of Administration
Cervical cancer	Topical
Gastrointestinal cancer	Oral; intravenous
Lung cancer	Inhaled; intravenous
Leukemia	Intravenous; extracorporeal
Metastatic carcinomas	Intravenous; oral
Brain cancer	Targeted; intravenous; intrathecal
Liver cirrhosis	Oral; intravenous
Psoriasis	Topical
Keloids	Topical
Baldness	Topical
Spinal cord injury	Targeted; intravenous; intrathecal
Parkinson's disease	Targeted; intravenous; intrathecal
Motor neuron disease	Targeted; intravenous; intrathecal
Alzheimer's disease	Targeted; intravenous; intrathecal

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a Therapeutic, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle

with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the Therapeutic, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the

quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The Therapeutics of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the Therapeutic of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed ~~in~~ the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20-500 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency

regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

5.13. DIAGNOSTIC UTILITY

deltex proteins, analogues, derivatives, and subsequences thereof, *deltex* nucleic acids (and sequences complementary thereto), anti-deltex antibodies, have uses in diagnostics. Such molecules can be used in assays, such as immunoassays, to detect, prognose, diagnose, or monitor various conditions, diseases, and disorders affecting deltex expression, or monitor the treatment thereof. In particular, such an immunoassay is carried out by a method comprising contacting a sample derived from a patient with an anti-deltex antibody under conditions such that immunospecific binding can occur, and detecting or measuring the amount of any immunospecific binding by the antibody. In a specific aspect, such binding of antibody, in tissue sections, preferably in conjunction with binding of anti-Notch can be used to detect aberrant Notch and/or deltex localization or aberrant levels of Notch-deltex colocalization in a disease state. In a specific embodiment, antibody to deltex can be used to assay in a patient tissue or serum sample for the presence of deltex where an aberrant level of deltex is an indication of a diseased condition. Aberrant levels of deltex binding ability in an endogenous Notch protein, or aberrant levels of binding ability to Notch (or other deltex ligand) in an endogenous deltex protein may be indicative of a disorder of cell fate (*e.g.*, cancer, etc.) By "aberrant levels," is meant increased or decreased levels relative to that present, or a standard level representing that present, in an analogous sample from a subject not having the disorder.

The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays,

complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few.

deltex genes and related nucleic acid sequences and subsequences, including complementary sequences, and other toporythmic gene sequences, can also be used in hybridization assays. *deltex* nucleic acid sequences, or subsequences thereof comprising about at least 8 nucleotides, can be used as hybridization probes. Hybridization assays can be used to detect, prognose, diagnose, or monitor conditions, disorders, or disease states associated with aberrant changes in deltex expression and/or activity as described *supra*. In particular, such a hybridization assay is carried out by a method comprising contacting a sample containing nucleic acid with a nucleic acid probe capable of hybridizing to *deltex* DNA or RNA, under conditions such that hybridization can occur, and detecting or measuring any resulting hybridization.

6. EXAMPLE: A MEMBER OF THE *NOTCH* GROUP OF INTERACTING LOCI, *DELTEX* ENCODES A CYTOPLASMIC BASIC PROTEIN

As described herein, we have proceeded with a molecular characterization of *Drosophila deltex*. We report the cloning and sequencing of *deltex*, and that *deltex* encodes a maternally and zygotically expressed transcript that results in the production of a basic protein of novel sequence. Expression of the *deltex* protein appears ubiquitous and at low levels within the cytoplasm. By ectopically expressing *deltex* under the control of a heatshock promoter, we confirm the cytoplasmic subcellular localization of deltex. Moreover, this overexpression does not result in any obvious phenotypic abnormalities in the fly. Finally, we examine genetically the functional effects of several *Suppressor of deltex* mutations upon the various *deltex* interactions. We demonstrate that in addition to suppressing all adult morphological defects of *deltex* alleles, these suppressors also are capable of suppressing most synergistic effects involving *deltex* and *Notch*, *Delta*, and *mastermind*.

6.1. RESULTS

6.1.1. MOLECULAR CLONING OF THE DELTEX LOCUS

The *deltex* allele dx^P was isolated during a P element-induced mutagenesis screen (Golubovsky, 1983, Dros. Inf. Ser. 59:42-43; Maine et al., 1985, Cell 43:521-529). *In situ* hybridization of P element sequences to polytene chromosomes revealed that the stock carrying the dx^P mutation ($dx^P sn3$) contained four P elements in regions 2A, 5C, 5D and 6A, respectively, on the X chromosome (data not shown). Because the cytological position of the *deltex* locus was previously assigned within the polytene interval 6A3,4 and 6F10,11 (Demerec et al., 1942, Yearbook-Carnegie Institution 41:191; Gorman and Girton, 1992, Genetics 131:99-112), these observations suggested that the *deltex* phenotype of dx^P was associated with the P element insertion at 6A.

We proceeded to isolate genomic DNA clones representing the 6A chromosomal region by constructing a genomic library of partial *Sau*3A digests of dx^P DNA and screening it with a P element DNA probe. The overlapping clones, lA25 and lA28, both of which carry P element homologous sequences (Figure 1A), were shown by *in situ* hybridization on polytene chromosomes to derive from the 6A region (data not shown). Using DNA fragments from these clones to screen a wild-type genomic library, we obtained three wild-type clones (lI1, lI2, lI3) that covered ~35 kb around the P element insertion site (Figure 1A). That the P element in 6A is causative of the *deltex* phenotype is suggested by an examination of genomic DNA from seven phenotypic revertants of dx^P, which were generated by genetic mobilization of the P element: In all cases the P elements were excised from the cloned region (data not shown).

6.1.2. TRANSCRIPTIONAL ACTIVITY OF THE 6A REGION

The transcriptional activity within the 35-kb cloned region was examined by Northern analysis: RNA samples from various developmental stages were probed with DNA fragments covering the entire cloned region. Only two transcription units were revealed by this analysis. The first one encoded a single size-class RNA of 1.1 kb that was detectable with both probes pdA8.5 and

pdA703 (Figure 1A). This RNA accumulated at high levels during all stages of development. Limited sequencing of a cDNA clone representing this transcription unit revealed homology to mammalian ribosomal protein L7a (not shown). The second transcription unit, detectable with probes pdA4.8 and pdA702 but not pdA703, encoded a single-sized RNA species of ~4 kb. Its accumulation profile appeared to be regulated throughout development (Figure 2): RNA was most abundant in adult females and during the first half of embryogenesis and accumulated at lower levels during the larval and pupal stages. The fact that the P element associated with the dx^P mutation was localized within this 4-kb transcript (see below) provided further evidence that this was the *deltex* transcription unit.

6.1.3. ALL *DELTEX* ADULT PHENOTYPES CAN BE RESCUED BY A 10-KB GENOMIC FRAGMENT

In order to confirm that this transcription unit encoded *deltex* sequences, we transformed *Drosophila* embryos with cloned DNA spanning coordinates ~+6 to ~-4 (Figure 1) and tested the ability of the introduced DNA to complement *deltex* mutations (see Section 6.3). The 10-kb *Xba*I genomic fragment used for this study contained the putative *deltex* transcription unit but interrupted the neighboring ribosomal protein transcription unit (Figure 1B). We obtained two independent transformant lines, denoted TX05B and TX012G, both of which carried insertions of the transposon on the second chromosome. In genetic complementation tests using all four *deltex* alleles, both TXO5B and TX012G rescued all the adult mutant phenotypes, including the wing, ocellar and rough eye defects (Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677). This rescue was complete for TXO5B, whereas in the case of TX012G, a small percentage of flies displayed a weak delta at the tip of the fifth longitudinal wing vein for all four *deltex* alleles (data not shown).

We previously reported that although *deltex* mutations are viable, about 40% of the eggs laid by homozygous *deltex* females (dx^{ENU}) failed to hatch (Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677). During the course of the present work it was noticed that this particular phenotype appeared more

variable than previously encountered: between 12 and 48% of dx^{ENU} embryos produced by homozygous dx^{ENU} females failed to hatch. Neither TX05B nor TXO12G improved this statistic: the exact same percentage of eggs failed to hatch whether or not their mutant mothers contained one copy of the transposon (see also below), suggesting that the observed lethality is not solely linked to *deltex* function.

Double mutant combinations between dx^{ENU} and the *Notch* allele *notchoid (nd)* are lethal (Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677). TXO5B and TXO12G are capable of rescuing this lethality: *nd* dx^{ENU} homozygous or hemizygous animals are rescued by one copy of the 10-kb *Xba*I transposon, although the transformant females are sterile (eggs are laid but never hatch). Two copies of the transposon do not improve this sterility. Additionally, the rescued animals display a wing phenotype (very thick veins ending in deltas and large nicks in the wing margin) very similar to that previously described for *nd* dx^{ENU}/*nd* + females (see Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677), though it is less severe with two copies of the transposon. On the one hand, these results clearly indicate that the 10-kb *Xba*I genomic fragment contains sequences encoding *deltex* information. On the other hand, they suggest that these transformant lines are failing to provide the entire adult complement of *deltex* function and/or that activity necessary for normal maternal function. It should be emphasized, however, that the semi-lethal phenotype associated with what appears to be *deltex* maternal function is not understood and the possibility that it reflects some aspect of the genetic background has not been excluded.

6.1.4. *DELTEX* ENCODES A BASIC PROTEIN RICH IN GLUTAMINE, HISTIDINE, AND SERINE RESIDUES

Two cDNA clones homologous to the 4-kb *deltex* transcript were isolated from an embryonic cDNA library (see Section 6.3) and their complete nucleotide sequence determined. These cDNAs, denoted cBE30 and cBE8, are 1299 bp and 2648 bp long, respectively, and overlap by 183 bp (Figures 1B,3). The composite nucleotide sequence (SEQ ID NO:1) is 3763 bp, which is a few

hundred base pairs shorter than the length of the RNA (~4 kb) as measured on RNA blots. Because a poly A tail and a poly A addition signal appear in the cDNA sequence, the 5′ extent of the *deltex* transcript appears not to be represented by the cDNAs. From limited sequencing of corresponding dx^P genomic DNA, we detect two short sequences approximately 150 bp and 300 bp upstream of the cDNA sequence that are highly homologous to a conserved sequence found at the cap site of other insect genes (Hultmark et al., 1986, Cell 44:429-438; Figure 3). There is no obvious TATA box, although parts of this region are A/T rich. Genomic sequencing of dx^P DNA also revealed two small introns of 594 and 68 bp, as well as the location of the P element insertion site to within the 5′ untranslated region (see Figure 1B).

Within the 3763-bp cDNA sequence there is a single open reading frame (ORF) of 2211 bp. The first AUG codon is within a sequence context that is consistent with the translation initiation consensus sequence determined for *Drosophila* (Cavener, 1987, Nucl. Acids Res. 15:1353-1361). Assuming that translation starts at that AUG, the *deltex* mRNA contains an untranslated leader sequence of at least 344 bp and an untranslated 3′ sequence of 1200 bp (Figure 3). The predicted protein product (SEQ ID NO:2) has 737 amino acids and an estimated molecular mass of 82 kDa (Figure 3). Computer database searches (see Section 6.3) failed to provide significant homologies to any known proteins.

The predicted deltex protein is rather basic, with an estimated pI of 9.8. One feature of this protein is the high percentage of the amino acids glutamine, histidine and serine: 11.26%, 5.97% and 11.94% respectively, compared to 5.0%, 2.8%, and 7.6% for the average *Drosophila* protein (Smoller et al., 1990, Genes Dev. 4:1688-1700). Most of the glutamine residues are concentrated in two clusters: the first one, extending between residues 261 and 302, is rich both in glutamine and histidine. The second one, starting at residue 488 and ending at residue 513, is composed mainly of glutamine. Such glutamine rich stretches are found in many proteins and are often referred to as *opa* or M repeats (Wharton et al., 1985, Cell 40:55-62; McGinnis et al., 1984, Nature 308:428-433). Their significance is unknown. Hydropathy plot analysis of the

protein sequence failed to identify a putative signal sequence or transmembrane domain (Kyte and Doolittle, 1982, J. Mol. Biol. 157:105-132).

6.1.5. THE DELTEX PROTEIN IS LOCALIZED WITHIN THE CYTOPLASM

To assess the spatial distribution and subcellular localization of the deltex protein, we raised mono- and polyclonal antibodies against bacterially expressed deltex fusion proteins (see Section 6.3). The specificity of the antibodies was routinely tested by immunoblot analysis and by immunofluorescent observation of Schneider 2 (S2) tissue culture cells (Schneider, 1972, J. Embryol. Exp. Morph. 27:353-365) transfected with *deltex* expression constructs. On immunoblots (not shown) the antibodies recognized an ~80 kDa protein, in agreement with that predicted from the DNA sequence.

The immunological staining of embryos and imaginal tissues with these antibodies revealed an apparently ubiquitous non-nuclear distribution. However, because this generalized pattern was also of low intensity, it presented difficulties in interpretation. To increase our confidence in this apparently cytoplasmic localization, we overexpressed the deltex protein in flies containing a P element transposon of *deltex* coding sequences under the control of the Hsp70 promoter (see Section 6.3). Using the antisera to compare the staining patterns of heat-shocked embryos containing or lacking the transposon, we observed a dramatic increase in staining intensity in embryos containing the transposon. Figure 4 shows that deltex is localized within the cytoplasm. However, because the Hsp70 promoter is ubiquitously expressed, we are not able to confidently assess the normal overall spatial pattern of deltex expression.

We examined further whether overexpression of deltex from this transposon was capable of rescuing *deltex* mutant defects and also whether additional phenotypic consequences might result from this over--and possibly ectopic--expression of deltex (see Section 6.3). Under the conditions we have tested, the $124A^{D}$ transposon is capable of completely rescuing, albeit with variable penetrance, the defects associated with *deltex* mutations. Additionally, various heat shock regimens of wild-type animals carrying the transposon revealed

no obvious phenotypic consequences of overexpression compared to control animals.

6.1.6. THE GENETIC SUPPRESSION OF *DELTEX*

During the course of characterizing *deltex* we recovered a spontaneous suppressor of *deltex*, which we denote $Su(dx)^{vp}$. Meiotic mapping placed this mutation on the second chromosome. This prompted us to examine two other second chromosome mutations, *Su(dx)* and $Su(dx)^2$, which were described by Morgan et al., 1931, Year book-Carnegie Institution 30:410 to suppress the adult phenotypes of *deltex* mutations.

Figure 5 summarizes the effects of these mutations on four *deltex* mutations, dx^{ENU}, dx^{SM}, *dx*, dx^{P}. All three *Su(dx)* mutations suppress in a dominant fashion the adult mutant phenotypes (wing, ocelli and eye) of these *deltex* alleles, although with differences in expressivity. *Su(dx)* and $Su(dx)^{vp}$ are the most efficient suppressors, suppressing completely the wing and ocellar defects of dx^{ENU} and dx^{SM}, and partially suppressing their rough eye phenotype. $Su(dx)^2$ has a weaker effect, suppressing only partially the wing phenotype of dx^{SM} (Figure 6).

Although the genetic behavior of the three second chromosome suppressors is similar with regard to *deltex*, the fact that they act in a dominant fashion and as homozygotes display no obvious mutant phenotype by themselves precludes us from concluding they are allelic. However, one line of genetic evidence to suggest that they may be allelic makes use of the observation that homozygous *Su(dx)* or $Su(dx)^{vp}$ animals in an $Ax^{E2}\,dx^{ENU}$ background do not survive. This contrasts with flies of the genotype $Ax^{E2}\,dx^{ENU}$; *Su(dx)*/+, which are viable (Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677). We find that any heterozygous combination of the suppressor mutations in combination with $Ax^{E2}\,dx^{ENU}$ results in lethality. Thus, these results are consistent with the notion that the three suppressor mutations are allelic.

6.1.7. SUPPRESSION OF THE PHENOTYPIC INTERACTIONS BETWEEN *DELTEX* AND *NOTCH, DELTA* AND *MASTERMIND*

Characteristic of the genetic behavior of *deltex* is its interactions with *Notch*, *Delta*, and *mastermind* (Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677). We were therefore interested in examining further the effects of the *Su(dx)* loci upon these interactions.

The *deltex* locus first caught our attention by virtue of its ability to suppress, in a dominant fashion, the lethality conferred by certain transheterozygous combinations of the *Abruptex* alleles of the *Notch* locus (*e.g.*, Ax^{E2}/Ax^{9B2}; Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677). This lethality is restored in the presence of *Su(dx)*, that is, animals that are Ax^{E2} dx^{ENU}/Ax^{9B2} +; *Su(dx)*/+ are no longer viable. Similarly, in the presence of *Su(dx)*, the normally lethal *nd* dx^{ENU} double mutation (Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677) now results in a viable combination, although these animals exhibit a severe loss of wing blade material such that only a narrow strip of blade remains (not shown).

A characteristic pupal lethality is displayed by *deltex* mutants bearing one copy of *N*, *Dl*, or *mam* (Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677). With *Notch* this lethality is complete, whereas with Dl and *mam* the condition is semi-lethal (Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677). We found that one copy of *Su(dx)* can partially suppress these interactions: a few animals of the genotype N^{54l9} dx^{ENU}/+ dx^{ENU}; *Su(dx)*/+ are able to escape lethality, although they display the same small, rough-eye defect as their dead siblings (see also Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677); animals that are dx^{ENU}/Y; *Su(dx)* +/+ mam^{IL115} or dx^{ENU}/Y; *Su(dx)*/+; Dl^{9P39}/+ are perfectly viable, but display clear *deltex* mutant phenotypes (ocelli and wing).

Previously, we reported that in the wing of $Ax^{E2}dx^{ENU}$ homozygous or hemizygous animals there is a suppression of the *Abruptex* mutant phenotype concomitant with an enhancement of the *deltex* mutant phenotype (Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677). As indicated above, animals homozygous for *Su(dx)* in this background do not survive. However, with one

copy of *Su(dx)* the wing defects are similar to those of Ax^{E2} single mutants. That is, with the addition of a single copy of *Su(dx)* there is a suppression of the effect of *deltex* upon Ax^{E2}. In addition, the triple mutants display a novel phenotype never observed in animals carrying any two of these mutations: many micro-and macrochaetae on the thoracic region are missing (Figure 7).

6.2. DISCUSSION

Genes that individually share similar mutant phenotypes or that combinatorially show a synergistic enhancement of a mutant phenotype are often regarded as integrated within a common developmental pathway. While not constituting proof of such integration, these observations nonetheless provide a powerful means for identifying potentially interacting components of complex developmental processes. This premise has formed the basis of genetic screens we have performed and has led to the identification of *deltex* as an interacting partner of *Notch*. Moreover, this finding has been strengthened by the demonstration that deltex also interacts with *Delta* and *mastermind*, two genes that not only share similar null phenotypes with those of *Notch*, but also display phenotypic interactions with *Notch* (Xu et al., 1990, Genes Dev. 4:464-475; Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677).

In this paper, we present our initial molecular characterization of the deltex locus. We have ultimately identified the *deltex* transcription unit by showing via germline-mediated transformation experiments that a 10-kb genomic fragment containing a ~5 kb transcription unit is capable of complementing most *deltex* mutant defects. Moreover, this genomic fragment rescues the normally lethal genetic interaction that results when flies are doubly mutant for *deltex* and *nd*. Finally, Northern analysis indicates a maternal loading of *deltex* transcripts into the developing oocyte, a finding that is consistent with the maternal effect observed upon embryogenesis in eggs laid by homozygous mutant mothers (Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677).

The phenotypic consequences of null mutations of *deltex* are not known. The only known deficiency ($Df(1)G4e^{L}H24i^{R}$) of the chromosomal region

in which *deltex* resides was generated synthetically (Stephenson and Mahowald, 1987, Dev. Biol. 124:1-8; see also Gorman and Girton, 1992, Genetics 131:99-112) and because of its size uncovers multiple loci. Moreover, the placement of a *Minute* locus within this deficiency has complicated the genetic analysis of *deltex*. *Minutes* exhibit a dominant haplo-insufficient phenotype of reduced fertility, long generation times and shortened and thin bristles. Numerous *Minute* loci are found within the *Drosophila* genome and in at least one case has been shown to encode a ribosomal protein gene (Kongsuwan et al., 1985, Nature 317:555-558; see also Burns et al., 1984, Mol. Cell. Biol. 4:2643-2652). In the course of our molecular characterization of *deltex*, we discovered a ribosomal protein (rp) transcription unit that resides ~2 kb from the 5' end of the *deltex* transcription unit. We have shown that P element transformation of a genomic fragment containing this rp transcription unit rescues fully the *Minute* phenotype of $Df(1)G4e^{L}H24i^{R}$. Thus, the proximity of the rp gene to *deltex* appears likely to have contributed to the difficulty in recovering *deltex* deficiencies (see also Lefevre, 1974, Cold Spring Harbor Symp. Quant. Biol. 38 591-599).

We have extended the genetic analysis of *deltex* by examining three second chromosome mutations that are effective suppressors of the *deltex* phenotype, though their allelism has not been established. That the relationship of *Su(dx)* to *deltex* and *Notch* reflects not a simple suppression of deltex is suggested by the synergistic effects that are displayed in triple mutants [*i.e.*; $Ax^{E2}dx^{ENU;}$ *Su(dx)*]: a novel phenotype of missing bristles is observed (Figure 7). Because homozygous $Ax^{E2}dx^{ENU}$ animals carrying a duplication of the *deltex* locus do not display this novel phenotype (not shown), the *Su(dx)* loci cannot be mere duplications of the *deltex* locus. Moreover, whereas animals homozygous for all three mutations are not viable, homozygotes for any two mutations are viable. Thus, there is the suggestion that the effects of *Su(dx)* mutations are the result of a three-way interaction between *Notch*, *deltex* and *Su(dx)*.

We have established by several criteria the specificity of the antibodies for the *deltex* protein, although immunological staining of tissues has been complicated by the low levels and apparently ubiquitous distribution of the

protein product. These results have been consistent with our efforts to detect the embryonic distribution of *deltex* mRNA *in situ* using appropriate nucleic acid probes. However, the use of a transposon to overexpress deltex in embryos has afforded us a view of deltex *in vivo* and to demonstrate a cytoplasmic distribution of the protein, in agreement with conceptual translation of the gene. Additionally, the ability of this transposon to rescue *deltex* mutant phenotypes without producing overt mutant consequences in wild-type animals is consistent with the evidence showing a generalized distribution of the gene product.

Two observations raise the possibility that the *in vivo* level of deltex protein accumulation is under translational control. First, in the 5′ non-coding region preceding the long ORF, there is an unusual arrangement of five ATG codons that are part of a short ORF of 14 codons (Figure 3). This organization is reminiscent of genes known to be under translational control (Abastado et al., 1991, Mol. Cell. Biol. 11:486-496; see also Kozak, 1986, Cell 44(2):283-292). Second, we have shown that in plasmid expression constructs, removal of these upstream initiator codons significantly increases the level of deltex protein accumulation in transfected S2 cells, as detected by immunofluorescence. Thus, this may account for the low levels of protein accumulation we detect *in vivo*, although artificially overexpressing deltex appears not to have any deleterious consequences (see above).

Biochemical and genetic studies have identified Delta and Serrate as ligands of Notch and have provided insights into the nature of their interactions with the Notch extracellular domain (Fehon et al., 1990, Cell 61:523-534; Rebay et al., 1991, Cell 67:687-699; PCT Publication No. WO 92/19737 dated November 12, 1992).

6.3. MATERIAL AND METHODS

6.3.1. ISOLATION OF NUCLEIC ACIDS

Isolation of *Drosophila* genomic DNA was performed as described by Preiss et al. (1988, EMBO J. 7:3917-3927). Total embryonic and larval RNAs from a Canton S strain were prepared according to the procedure of

Chirgwin et al. (1979, Biochemistry 18(24)5294-5299). Pupal and adult RNAs were generously provided by A. Preiss (Preiss et al., 1988, EMBO J. 7:3917-3927). Poly $(A)^+$ RNA was selected by serial passage over oligo(dT)- cellulose (Stratagene) according to Maniatis et al. (1989, Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, Cold Spring Harbor Laboratory, New York). ^{32}P-labelled DNA probes were prepared by random oligonucleotide priming (Feinberg and Vogelstein, 1984, Anal. Biochem. 137:266-267). Enzymes were used as instructed by the manufacturers. All other procedures were carried out according to standard protocols (Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York).

The genomic library of dx^P DNA was prepared as follows: 60 mg of DNA from dx^P flies were digested with 2 units of *Sau*3A in a reaction volume of 320 ml for 1 h, and then fractionated by centrifugation on a continuous 10-40% sucrose gradient for 22 h at 22,000 rpm (rotor SW40, Beckman). Fractions containing DNA of 15 kb or more were collected, dialysed, precipitated with ethanol, and ligated to 1 mg of *BamH*I cut arms of λEMBL3 (Frischauf et al., 1983, J. Mol. Biol. 170:827-842) in a reaction volume of 10 ml. Half of the ligation mixture was used in a standard *in vitro* lambda packaging reaction (kit from Amersham), yielding a total of 2 x 10^6 recombinant phages. The *Drosophila* wild-type genomic library in λEMBL3 was provided by J. Tamkum (Tamkun et al., 1992, Cell 68:561-572). The cDNAs cBE8 and cBE30 were recovered from a λgt10 cDNA library made from 3-12 h embryonic mRNA and donated by Larry Kauvar (Poole et al., 1985, Cell 40:37-43).

6.3.2. P-MEDIATED TRANSFORMATION

For the genomic rescue experiments, a 10-kb *Xba*I genomic fragment (Figure 1) encompassing the ~5-kb *deltex* transcription unit was cloned into the *Xba*I site of the P transformation vector pDM23, which carries the *rosy* gene as a selection marker (G. Rubin, unpublished). This DNA was co-injected with the Δ2-3 helper plasmid (to provide transposase; Robertson et al., 1988,

Genetics 118:461-470) into *cn; ry*^{506} embryos using essentially the procedure described by Spradling (1986, in Glover, D.M. (ed.), *Drosophila - A Practical Approach*, IRL Press, Oxford, pp. 175-197). Three phenotypically *cn* and *ry*$^{+}$ G1 females were recovered and mated to *cn; ry*^{506} males. One of the insertions was X-linked and male lethal and not used further. The other two insertions were localized to chromosome 2 and single lines were established using the balancer strain. *Bc Elp/CyO; ry506.*

For experiments involving overexpression of *deltex in vivo*, we used the pCaSpeR-hs transformation vector (Thummel and Pirrotta, 1991, DIS 71:150), which was kindly provided by C. Thummel. cDNA sequences extending from 33 nucleotides 5′ of the translational start site to a sole *Spe*I site within the 3′ non-coding region were ligated into the *EcoR*I-*Xba*I digested vector. DNAs were injected into W^{1118} embryos ; 9 w^{+} transformant fly lines were recovered, one of which, 124A^{D},was chosen for this study. When crossed to *deltex* mutants, this transgenic line significantly improved *deltex* mutant defects in the absence of heat shock and produced wild-type appearing flies with the application of daily 1 hour heat shocks at 37°C. For the immunofluorescent staining pictured in Figure 4 (see also below), we applied the following heat shock regimen to an overnight collection of embryos produced from homozygous 124A^{D} flies: 50 min at 37°C, 50 min at 25°C, 50 min at 37°C. After a 4 hour recovery at 25°C, the embryos were fixed in 4% paraformaldehyde/phosphate-buffered saline (PBS) solution.

6.3.3. SEQUENCE DETERMINATION AND ANALYSIS

The *EcoR*I-cDNA insert from cBE8 was subcloned directly in both orientations into Bluescript KS-, M13mp18 and M13mp19 vectors. Overlapping deletions were produced on the inserts using the Exonuclease III-Mung Bean Nuclease system (kit from Stratagene). Additional deletions were obtained through the use of restriction sites within the vector polylinker and the cDNA inserts. The cDNA insert of cBE30 contains two internal *EcoR*I sites but no *Hind*III site: it was cloned into M13mp18 and M13mp19 vectors as three separate *EcoR*I pieces, and into Bluescript KS- vector as a *Hind*III fragment

containing sequences from λgt10 on both sides. Single-stranded cDNAs were produced according to the manufacturer's instructions. Both strands of the cDNAs were sequenced using the dideoxynucleotide chain termination method (Sanger et al., 1977, Proc. Natl. Acad. Sci. USA 74:5463-5467) and the Sequenase kit (U.S. Biochemical Corp.), with the M13 universal and reverse primers. cDNA sequences that were not accessible by these methods were obtained using synthetic primers complementary to part of a previously determined sequence. Genomic sequences of interest from phages A25 or I2 were subcloned into convenient restriction sites of Bluescript KS- and sequenced using synthetic primers.

DNA sequence manipulations were performed using Intelligenetic's PC-GENE software. Open reading frame prediction and plotting were performed using the University of Wisconsin program CODONPREFERENCE (Gribshov et al., 1984, Nucl. Acids Res. 12:539-549). The GenPept and SWISS-PROT databases were searched with all or part of the deduced amino acid sequence using the FASTA program (Pearson and Lipman, 1988, Proc. Natl. Acad. Sci. USA, 85:2444-2448) available by the GenBank FASTA server through BITNET.

6.3.4. EXPRESSION CONSTRUCTS AND IMMUNOLOGICAL PROCEDURES

cDNA representing the entire *deltex* coding region was inserted into the pGex1 expression vector and expressed in *E. coli* XL1-Blue (see Smith and Johnson, 1988, Gene 67:31-40). The bacterial pellet was washed first in ice-cold 50 mM Tris pH 8.0, 50 mM EDTA, 50 mM NaCl before being resuspended in ice-cold 50 mM Tris pH 8.0, 150 mM NaCl, 1 mM EDTA, 1 mg/ml lysozyme, 1 mM phenylmethylsulfonylfluoride (PMSF). After ~1 hour on ice, the cells were lysed by the addition of Triton X-100 to 1% final concentration. The lysate was sonicated briefly to shear DNA and then cleared by centrifugation. After overnight incubation at 4°C with glutathione-agarose beads (Sigma), the fusion protein was eluted from the PBS-washed beads with 5 mM reduced glutathione, 50 mM Tris pH 8.0 (see Smith and Johnson, 1988, Gene 67:31-40). Standard procedures were used to immunize Sprague-Dawley rats with soluble

fusion protein (Harlow and Lane, 1988, *Antibodies: A Laboratory Manual*, Cold Spring Harbor, Cold Spring Harbor Laboratory, New York). Immune sera was tested for positive reactivity by immunofluorescent staining of transiently-transfected S2 tissue culture cells (see Fehon et al., 1990, Cell 61:523-534) and by immunostaining of protein blots (Harlow and Lane, 1988, *Antibodies: A Laboratory Manual*, Cold Spring Harbor, Cold Spring Harbor Laboratory, New York). The expression vector used in the transfections was pRmHa-3 (Bunch et al., 1988, Nucl. Acids Res. 16:1043-1061), into which *deltex* cDNA sequences were inserted. Hybridoma fusions were performed by the Howard Hughes Medical Institute's Hybridoma Facility at Yale University. The antibody supernatant used in this study derived from the cell line C645.17A. Immunofluorescent staining and confocal imaging of embryos was essentially as described by Fehon et al. (1991, J. Cell Biol. 113:657-669).

6.3.5. STRAINS AND CROSSES

Stocks were maintained and crosses were performed using standard procedures described previously (Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677). Genetic markers and strains not specifically mentioned here can be found in Lindsley and Grell (1968, Genetic Variations of Drosophila melanogaster, Publication number 627, Carnegie Institute of Washington, Washington D.C.) or Lindsley and Zimm (1985, Dros. Inf. Serv. 62), Lindsley and Zimm (1986, Dros. Inf. Serv. 64), Lindsley and Zimm (1987, Dros. Inf. Serv. 67), and Lindsley and Zimm (1990, Dros. Inf. Serv. 68).

6.3.6. PHENOTYPIC REVERSION OF DX^P

Homozygous dx^P sn^3 females were mated to *cn*; $P(ry^+; D2\text{-}3)$ ry^{506} *Sb/TM6* males, which provide a stable source of P transposase (Robertson et al., 1988, Genetics 118:461-470). dx^P sn^3/Y; *cn/+*; $P(ry^+;D2\text{-}3)$ ry^{506} *Sb/+* F1 males were then mated to *C(1)A, y* females. From ~3000 males scored in the F2 generation, we recovered seven phenotypically wild-type males. These were mated to *C(1)A, y* females to establish single lines and their genomic DNA was

analyzed further by standard Southern blot procedures (Maniatis et al., 1989, Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, Cold Spring Harbor Laboratory, New York)

6.3.7. COMPLEMENTATION OF *DELTEX* ALLELES BY THE DELTEX TRANSPOSONS TXO5B AND TXO12G

y dx^{ENU} sn^3, dx^{SM} t^2 *v*, *ec dx* and dx^P sn^3 females were mated to *cn* TXO5B/*cn* +; ry^{506} males and to *cn* TXO12G/*cn* +; ry^{506} males to assess effects on adult phenotypes. To assess the effects on embryonic lethality, *y* dx^{ENU} sn^3 females were crossed to *y* dx^{ENU} sn^3/*Y; cn* TXO5B/+ males and to dx^{ENU} sn^3/*Y; cn* TXO12G/+ males; dx^{SM} t^2 *v* females were crossed to dx^{SM} t^2 *v*/*Y; cn* TXO12G/+ males. From this cross both sibling progeny containing or lacking the transposon were mated to males of identical X chromosome genotype and the percentage of unhatched embryos was calculated.

6.3.8. ORIGIN OF *SU(DX)*^{SP}

The mutation (*Su(dx)*^{sp}) was found in a homozygous Ax^{E2} dx^{ENU} stock maintained in our laboratory and was shown to segregate with the second chromosome. The following crosses were performed: An Ax^{E2} dx^{ENU}; *Su(dx)*^{sp}/+ female was mated to *FM6/Y; Adv/SM1* males. Individual F1 Ax^{E2} dx^{ENU}/*FM6*; *Su(dx)*^{sp}/*SM1* or Ax^{E2} dx^{ENU}/*FM6*; +/*SM1* females were mated to individual F1 Ax^{E2} dx^{ENU}/*Y; Su(dx)*^{sp}/*SM1* males. The individual F2 Ax^{E2} dx^{ENU}/Ax^{E2} dx^{ENU}; *Su(dx)*^{sp}/*SM1* females were then mated to individual Ax^{E2} dx^{ENU}/*Y; Su(dx)*^{sp}/*SM1* males to establish stock lines. No homozygous *Su(dx)*^{sp} animals were found in the F3 offsprings from these crosses, indicating that the triple homozygous mutants were lethal. *Su(dx)*^{sp} homozygotes and *Su(dx)*^{sp}/*Su(dx)* heterozygotes were viable and displayed no obvious mutant phenotypes.

6.3.9. CROSSES BETWEEN *SU(DX)* MUTATIONS AND DELTEX ALLELES

y dx^{ENU} sn^3, dx^{SM} l^2 *v, ec dx* and dx^P sn^3 females were mated to $Su(dx)^{vp}$, *Su(dx)* and *ed* $Su(dx)^2$ males to observe double mutant effects of *dx* and *Su(dx)*. *y* dx^{ENU} sn^3; *+/SM1* females were mated to *y* dx^{ENU} sn^3/*Y; Su(dx)/+* males. F1: *y* dx^{ENU} sn^3; *Su(dx)/SM1* females x *y* dx^{ENU} sn^3/*Y; Su(dx)/SM1* male. F2: *y* dx^{ENU} sn^3; *Su(dx)* females x *y* dx^{ENU} sn^3/*Y; Su(dx)* males.

6.3.10. CROSSES BETWEEN *SU(DX)* AND MUTATIONS OF THE *NOTCH* LOCUS

y w Ax^{9B2}/*FM7C* x Ax^{E2} dx^{ENU}/Y; *Su(dx)/SM1*. F1 female progeny: *y w* Ax^{9B2}/Ax^{E2} dx^{ENU}; *Su(dx)/+*, *y w* Ax^{9B2}/Ax^{E2} dx^{ENU}; *SM1/+*, *FM7C*/Ax^{E2} dx^{ENU}; *Su(dx)/+*, *FM7C*/Ax^{E2} dx^{ENU}; *SM1/+*.

y Ax^{9B2} *sn3* x Ax^{E2} dx^{ENU}/Y; $Su(dx)^{vp}$/*Adv*. F1 female progeny: *y* Ax^{9B2} sn^3/Ax^{E2} dx^{ENU}; *Su(dx)/+* and *y* Ax^{9B2} sn^3/Ax^{E2} dx^{ENU}; *SM1/+*.

y w Ax^{9B2}/*FM7C* x Ax^{E2} dx^{ENU}/Y. F1 female progeny: *y w* Ax^{9B2}/Ax^{E2} dx^{ENU} and *FM7C*/Ax^{E2} dx^{ENU}.

Ax^{E2} dx^{ENU} females x $Su(dx)^{vp}$, *Su(dx)* or *ed* $Su(dx)^2$ males.

w^a *nd* dx^{ENU} sn^3/*FM7C* x *Su(dx)*. F1 male progeny: w^a *nd* dx^{ENU} sn^3/Y; *Su(dx)/+* and *FM7C/Y; Su(dx)/+*.

w^a *nd* dx^{ENU} sn^3/*FM7C* x $Su(dx)^{vp}$/Y. F1 male progeny: w^a *nd* dx^{ENU} sn^3/Y; $Su(dx)^{vp}$/+ and *FM7C*/Y; $Su(dx)^{vp}$/+.

nd^2 dx^{ENU} sn^3/*FM7C* x *Su(dx)*/Y. F1 male progeny: nd^2 dx^{ENU} sn^3/Y; *Su(dx)/+* and *FM7C*/Y; *Su(dx)/+*.

nd^2 dx^{ENU} sn^3/*FM7C* x $Su(dx)^{vp}$/Y. F1 male progeny: nd^2 dx^{ENU} sn^3/Y; $Su(dx)^{vp}$/+ and *FM7C*/Y; $Su(dx)^{vp}$/+.

nd^2 dx^{ENU} sn^3/*FM7C* x *ed* $Su(dx)^2$/Y. F1 male progeny: nd^2 dx^{ENU} sn^3/Y; *ed* $Su(dx)^2$/+ and *FM7C*/Y; *ed* $Su(dx)^2$/+.

y w^a N^{54l9} dx^{ENU} sn^3/*FM7C* x *y* dx^{ENU} sn^3/Y; *Su(dx)*. F1 female progeny: *y* w^a N^{54l9} dx^{ENU} sn^3/y + + dx^{ENU} sn^3; *Su(dx)/+*, *FM7C* /*y* dx^{ENU} sn^3; *Su(dx)/+* and *y* w^a N^{54l9} dx^{ENU} sn^3/*FM7C*; *Su(dx)/+*.

Ax^{E2} sn^3; *Su(dx)^{sp}/SM1* females x *ed Su(dx)^2*, *Su(dx)* and *Ax^{E2} sn^3*/Y; Su(dx)^{sp}/SM1 males.

6.3.11. CROSSES INVOLVING *SU(DX)*, *DELTEX*, *DELTA* AND *MASTERMIND*

y dx^{ENU} sn^3; *Su(dx)* females x *Dl^{9P39}/TM1* males. F1 male progeny: *y dx^{ENU} sn^3*/Y; *Su(dx)*/+; *Dl^{9P39}*/+ and *y dx^{ENU} sn^3*/Y; *Su(dx)*/+; *TM1*/+.

y dx^{ENU} sn^3; *Su(dx)* females x *cn bw sp mam^{IL115}/Cyo* males. F1 male progeny: *y dx^{ENU} sn^3*/Y; *Su(dx)* + + + +/+ *cn bw sp mam^{IL115}* and y *dx^{ENU} sn^3*/Y; *Su(dx)/Cyo*.

6.3.12. CROSSES COMPARING *SU(DX)* MUTATIONS

Ax^{E2} dx^{ENU}; *Su(dx)^{sp}/Cyo* females x *Ax^{E2} dx^{ENU}*/Y; *Su(dx)^{sp}/Cyo*.

Ax^{E2} dx^{ENU}/FM7C; *Su(dx)/SM1* females x *Ax^{E2} dx^{ENU}*/Y; *Su(dx)/SM1*.

Ax^{E2} dx^{ENU}; *Su(dx)^{sp}/Cyo* females x *Ax^{E2} dx^{ENU}*/Y; *Su(dx)/SM1*. F1 progeny: *Ax^{E2} dx^{ENU}*/Y; *Cyo* or *SM1/Su(dx)^{sp}* or *Su(dx)*, *Ax^{E2} dx^{ENU}/Ax^{E2} dx^{ENU}*; *Cyo* or *SM1/Su(dx)^{sp}* or *Su(dx)*.

Ax^{E2} dx^{ENU}; *Su(dx)^{sp}/Cyo* females x *ed Su(dx)^2* males. F1 progeny: *Ax^{E2} dx^{ENU}*/+ +; *Su(dx)^{sp}/ed Su(dx)^2*, *Ax^{E2} dx^{ENU}*/Y; *Cyo/ed Su(dx)^2*, *Ax^{E2} dx^{ENU}/Ax^{E2} dx^{ENU}*; *Cyo/ed Su(dx)^2*.

6.3.13. CROSSES INVOLVING DUPLICATIONS OF THE *DELTEX* LOCUS

y w^a N^{5419}/FM6 females x Tp(1:Y) J104 *y B^s; Yy^+*(6E; Y^s) males. F1: *y w^a N^{5419}*/Tp(1:Y) J104 *y B^s; Yy^+*(6E; Y^s) females x *FM7C/Y* males. F2: Tp(1:Y) J104 *y w^a $N^{5419}B^s$; Yy^+*(6E; Y^s)/*FM7C* individual females x *Ax^{E2} dx^{ENU}*/Y males. F3: Tp(1:Y) J104 *y w^a $N^{5419}B^s$; Yy^+*(6E; Y^s)/*Ax^{E2} dx^{ENU}*/Y virgins x *Ax^{E2} dx^{ENU}*/Y males. F4: *Ax^{E2} dx^{ENU}/Ax^{E2} dx^{ENU}*/DpJ104 *y w^a $N^{5419}B^s$* (1A; 6E) animals.

7. DELTEX IMPLICATED IN NOTCH-MEDIATED SIGNAL TRANSDUCTION: EVIDENCE FOR CYTOSOLIC INTERACTION WITH NOTCH CDC10/SWI6/ANKYRIN REPEATS

As described herein, we have demonstrated a direct interaction between the novel cytoplasmic protein encoded by the *deltex* locus and the transmembrane receptor encoded by *Notch*, by expression studies conducted in cultured cells, in yeast, and in the imaginal wing disc. deltex binds specifically to the Notch cdc10/SWI6/ankyrin ("ANK") repeats, a region that is crucial for Notch signaling and that constitutes the most conserved domain among Notch family members. In addition, we describe a new class of viable *Notch* allele that is associated with a missense mutation within the ANK repeat region and that, like mutations of *deltex*, behaves as a dominant intragenic suppressor of the *Abruptex* 'negative complementation'.

7.1. DELTEX INTERACTS WITH NOTCH INTRACELLULAR DOMAIN

We explored the possibility of protein-protein interactions between deltex and Notch by examining the relative subcellular localization of the two proteins after co-expressing them in *Drosophila* Schneider 2 (S2) cultured cells (Schneider, 1972, J. Embryol. Exp. Morph. 27:353-365). S2 cells were co-transfected with plasmid expression constructs that placed *Notch* and *deltex* under the inducible control of the *Drosophila* metallothionein and *Hsp70* promoters, respectively. S2 cells do not express endogenous Notch (Fehon et al., 1990, Cell 61:523-534) and express deltex at levels too low for reliable immunofluorescent detection (see Section 6 hereinabove and data not shown). Notch expression was induced prior to heat-shock induction of deltex to ensure proper cell surface localization of Notch. By aggregating cells expressing both Notch and deltex with cells expressing Delta, a presumptive membrane-bound ligand of Notch (Fehon et al., 1990, Cell 61:523-534; Heitzler and Simpson, 1991, Cell 64:1083-1092), a dramatic co-capping of these proteins was induced at the point of cellular contact. Fig. 8a shows co-localization between deltex and Notch, indicating molecular interaction between the two proteins. Moreover, this co-localization

was evident even in the absence of capping with Delta, although in this case Notch and deltex were mis-localized within peri-nuclear regions of the cell (Fig. 8b). This latter result suggests that there is no ligand dependency of deltex association with Notch.

When the same aggregation experiment was performed using plasmids expressing deltex in combination with others expressing either Delta or Serrate (another presumptive ligand of Notch; Rebay et al., 1991, Cell 67:687-699), no co-localization was observed between deltex and these transmembrane proteins (Fig. 8c, d). This indicates that overexpression itself is not causing deltex to concentrate in the regions of cellular contact where Notch and Delta accumulate (Fehon et al., 1990, Cell 61:523-534). Moreover, the results indicate that the genetic interaction previously noted between *deltex* and *Delta* (Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677) is likely an indirect reflection of a direct Notch/deltex interaction.

Fig. 8 Methods: Cell culture conditions and media were as described (Fehon et al., 1990, Cell 61:523-534). For transfections, cells were transferred in fresh media to 6-well plates (Falcon) and allowed to attach to plastic for 1-3 h (cell density ~3/4 confluent). Ten micrograms total plasmid DNA in 0.5 ml serum-free media (SFM) were mixed with 0.5 ml SFM containing 50 ml LipofectACE™ (Life Technologies, Inc.). After 15-30 min, mixture was added to cells previously rinsed with SFM. After ~6 h incubation, transfection mixture was replaced with media plus serum. Expression from the metallothionein promoter was induced ~24 h later by $CuSO_4$ addition as described (Fehon et al., 1990, Cell 61:523-534), followed 12-18 h later with induction of the *Hsp 70* promoter that consisted of two 30 min incubations at 37°C with an intervening 30 min at 25°C. Three to five hours later, cells were incubated with anti-Notch antibody for 30 min and fixed in PLP fixative (Tomlinson and Ready, 1987, Dev. Biol. 120:336-376). Antibody incubations and cell mounting were as described (Fehon et al., 1990, Cell 61:523-534) using anti-deltex antibodies followed by FITC- and Texas Red-conjugated goat anti-mouse and anti-rat antibodies (Jackson ImmunoResearch Laboratories, Inc.). Third instar larvae

from transgenic line *124A*^{D} (see Section 6) were heat shocked as above to induce deltex expression and dissected wing discs were fixed and incubated with antibodies as outlined above. Confocal images were obtained as described (Xu et al., 1992, Development 115:913-922); cropping and pseudo-coloring were performed using Adobe Photoshop (Adobe Systems, Inc.) computer program.

7.2. NOTCH AND DELTEX CO-LOCALIZATION *IN VIVO*

The above demonstration of Notch/deltex interaction in transfected cells prompted us to explore whether such interaction was detectable *in vivo*. We examined the imaginal wing disc, a tissue that requires both proteins for its proper development (Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677). In addition, Notch displays a polarized distribution to the apical surface of the disc epithelium (Fehon et al., 1991, J. Cell Biol. 113:657-669). Because the endogenous level of deltex protein accumulation is below the threshold necessary for confident immunofluorescent detection (see Section 6 hereinabove), we used a transformant fly line carrying an inducible deltex construct to elevate transiently the *in vivo* pool of deltex protein. Over- and/or ectopic-expression from this transposon rescues *deltex* mutant defects and has no obvious phenotypic consequences in otherwise wild-type animals (see Section 6 hereinabove). Fig. 8e shows a coincidence of deltex and Notch proteins at the apical surface of the wing disc epithelium, confirming the co-localization data of the S2 cell culture assay and suggesting that Notch/deltex interactions normally occur *in vivo*.

7.3. NOTCH ANKYRIN REPEATS ARE BOTH NECESSARY AND SUFFICIENT FOR DELTEX ASSOCIATION

The 938-amino-acid intracellular domain of Notch contains several structural motifs (Stifani et al., 1992, Nature Genetics 2:119-127; Wharton et al., 1985, Cell 43:567-581; Kidd et al., 1986, Mol. Cell. Biol. 6:3094-3108; Breeden and Nasmyth, 1987, Nature 329:651-654). To identify those regions involved in deltex binding, we expressed deltex along with a set of Notch deletion constructs (Fig. 9a) in the S2 cell culture assay. The phenotypic consequences of expressing

these same deletions of Notch were also recently examined in transgenic flies (Rebay et al., 1993, Cell 74:319-329). The constructs Δ B-S and Δ S-S, which delete sequences C-terminal of the ANK repeats, did not visibly impede deltex association (Fig. 8f, g). Overexpression of these deletions within the fly produced only mild phenotypes similar to those resulting from overexpression of an intact version of Notch (Rebay et al., 1993, Cell 74:319-329). This stands in contrast to the results obtained with deletions that removed the ANK repeats (ECN or Δ cdc10). In the S2 cell assay, we detected no association of deltex with these versions of Notch (Fig. 8h, i). In the fly, overexpression of both the ECN and Δ cdc10 constructs resulted in severe dominant-negative phenotypes, demonstrating an essential role for the ANK repeats in Notch signal transduction (Rebay et al., 1993, Cell 74:319-329). Combined, these results not only implicate the ANK repeats in mediating Notch/deltex interaction (see below), but also suggest a role for deltex in intracellular signaling events.

To determine whether the ANK repeats alone were sufficient to promote deltex binding, we produced an expression construct, pMTDl/NANK, that affixed the Notch ANK repeats to the cytoplasmic domain of Delta (Fig. 9b). Because Delta shows no physical association with deltex (Fig. 8c), co-localization of the two proteins at the cell surface would be a consequence of the Notch ANK repeats. Fig. 8, panels j and k show this to be the case. Indeed, the deltex/Notch-ANK-repeat interaction is emphasized in Fig. 8k, which is an electronic merging of the side-by-side image displayed in Fig. 8j. Unlike the staining patterns obtained from other transfection experiments, this shows that the co-localization is offset, *i.e.*, not coincident, reflecting the labeling of Notch in one cell and that of deltex (through hybrid Delta/Notch-ANK-repeat protein) in an adjacent cell. Thus, the Notch ANK repeats are both necessary and sufficient for deltex binding to occur.

Because ANK repeats, in general, are a conserved feature of many proteins, we sought to address the specificity of deltex binding for those repeats of Notch. We replaced the ANK repeats of pMTDl/NANK with those of the *Drosophila* gene, *cactus*, to produce the expression plasmid, pMTDl/CANK

(Fig. 9b). *cactus* encodes an I-κ B cognate that has been shown to bind, via ANK repeats, a *Drosophila* NF-κ B cognate, dorsal (Geisler et al., 1992, Cell 71:613-621; Kidd, 1992, Cell 71:623-635). In the S2 cell expression assay, no association of deltex for the ANK repeats of cactus was observed (Fig. 8l), although immunoblot analysis indicated a hybrid Delta/cactus protein of proper size was expressed in these cells (Fig. 9c). This result indicates a specific association of deltex for the ANK repeats of Notch and serves as a control for the concern that overexpression of ANK repeats, *per se*, promotes deltex association.

Fig. 9 Methods: All constructs were based on the expression vector pRmHa-3 (Bunch et al., 1988, Nucl. Acids Res. 16:1043-1061), which uses the inducible *Drosophila* metallothionein promoter to drive expression. a, Details of plasmid construction have been described (Rebay et al., 1993, Cell 74:319-329). b, Encoded Notch ANK repeats were isolated as a PCR-derived DNA fragment containing artificial *Nde*I and *Bsp*EI sites. The following synthetic oligonucleotide primers (H.H.M.I. facility, Yale University) were used: 5′ GCG CAT CAG GAT CAT ATG AAG CAC GAT GTG GAT GCA 3′ (SEQ ID NO:20) and 5′ GGC CAC ATC GTC CGG AAA TCG ATC CAT GTG ATC 3′ (SEQ ID NO:21). To generate pMTDl/NANK, the following three-piece ligation was performed: a 2.6-kb *Eco*RI-*Nde*I DNA fragment from pMTDl1 (Fehon et al., 1990, Cell 61:523-534) and the *Nde*I/*Bsp*EI-digested PCR fragment were inserted into the *Eco*RI and *Xma*I sites of pRmHa-3. The resulting construct encoded Notch protein sequences beginning four residues before the first ANK repeat and ending nine residues after the sixth ANK repeat. For pMTDl/CANK, the same three-piece ligation was performed except a 0.7-kb *Ase*I-*Bsp*EI DNA fragment from the *cactus* cDNA pcact5B (Geisler et al., 1992, Cell 71:613-621) replaced the fragment encoding the Notch ANK repeats. The resulting construct encoded eight residues of *cactus* before the first ANK repeat and five residues of *cactus* after the sixth ANK repeat. For both constructs, a TAG stop codon was provided by the *Xba*I site within the vector polylinker sequence. c, Standard procedures (Harlow and Lane, 1988, *in* Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York) were used for the preparation of cell

lysates, PAGE-electrophoresis (3-15% gel) and immunoblot analysis. The anti-Delta monoclonal antibody C584.9B was used in conjunction with goat anti-mouse peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, Inc.) and the LumiGLO™ (Kirkegaard & Perry Laboratories, Inc.) chemiluminescent detection kit according to manufacturer's instructions.

7.4. DIRECT NOTCH/DELTEX INTERACTION INDICATED BY YEAST EXPRESSION STUDIES

The above demonstration of a specific association between deltex and Notch ANK repeats leaves open the question of whether the interaction is mediated directly or through other cellular components. To address this question, we have conducted similar expression studies in yeast using the so-called 'interaction trap' technique (Zervos et al., 1993, Cell 72:223-232). The "interaction trap" system involves three plasmids co-transfected in a yeast cell. The first plasmid is designed to express a fusion protein with a DNA binding domain of a yeast transcription factor LexA. The second plasmid is designed to produce a fusion protein with an acidic activation transcription domain (ACT). The third plasmid is a *lacZ* reporter gene which is activated by the binding of functional LexA protein to its upstream elements. Since the LexA fusion protein does not have an acidic transcription activation domain, it cannot activate the *lacZ* reporter gene. However, if there is a protein-protein interaction between a LexA fusion and an ACT fusion, a functional LexA protein is reconstructed which activates *lacZ* reporter gene transcription (resulting in synthesis of β-galactosidase).

Two expression plasmids were constructed. One (LexA-Notch ICN1) encoded a LexA DNA-binding domain fused to a portion of the Notch intracellular domain (amino acids 1827-2258; Wharton et al., 1985, Cell 43:567-581) that included the ANK repeats. The other plasmid (pJG4-5-deltex) encoded the entire deltex protein fused to an acidic transcription activation domain (ACT-deltex). These were co-transformed into yeast carrying a reporter gene (*LexAoperator-lacZ*) plasmid. Notch/deltex interactions would be expected to mediate the formation of a complex between the LexA-Notch ICN1 and ACT-

deltex proteins resulting in the restoration of transcriptional activity. This would be detected as a blue yeast colony due to induced β-galactosidase synthesis. As presented in Fig. 10, a specific interaction was indeed detected between Notch and deltex within the yeast cell. This result suggests that the Notch/deltex interaction observed within *Drosophila* cells is the consequence of a direct protein-protein interaction.

Fig. 10 Methods: The 'interaction trap' method was as described (Zervos et al., 1993, Cell 72:223-232). R. Finley and R. Brent generously provided yeast strain EGY40 and plasmids LEX202+PL (to make *LexA* fusions), pJG4-5 (to make acidic activation domain fusions), pSH18-34 (*LexAop-lacZ* reporter gene) and pRFHMI (*LexA-bicoid* fusion). The entire *groucho* coding region (Delidakis et al., 1991, Genetics 129:803-823) was isolated by PCR and inserted into the *Eco*RI restriction sites of LEX202+PL and pJG4-5 to create LexA-groucho and pJG4-5-groucho. A PCR-derived DNA fragment containing artificial *Eco*RI and *Sal*I sites and encoding amino acids 1827-2258 of Notch was inserted into LEX202+PL to create LexA-Notch ICN1. The entire coding region of *deltex* was recovered by PCR and inserted into the *Xho*I site of pJG4-5 to create pJG4-5-deltex. Yeast transformations were performed as described (Gietz et al., 1992, Nucl. Acids Res. 20:1425).

7.5. INTRAGENIC SUPPRESSOR MUTATION MAPS WITHIN ANK REPEAT

In the same genetic screen (Xu et al., 1990, Genes Dev. 4:464-475; Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677) that identified *deltex* as a potentially interacting partner of *Notch*, an unusual allele of Notch was also recovered. This allele, denoted N^{Su42c}, is homozygous viable, and like mutations of *deltex*, *Delta* or *mastermind*, suppresses completely the pupal lethality associated with the Ax^{E2}/Ax^{9B2} 'negative complementation'.

Intra-genic recombination analyses indicated that the N^{Su42c} lesion was positioned centromere-proximal, and thus 3', to the Ax^{9B2} mutation located within the genomic region encoding the 23rd EGF-like repeat. We have

sequenced Ax^{9B2} *su42c* genomic DNA encompassing the 17th EGF-like repeat to the C-terminal end of the protein and find a missense mutation that results in an alanine to valine substitution within the fifth ANK repeat (Fig. 11). This alanine is conserved among all *Notch* homologs in mice, rats, humans and *Xenopus* (Coffman et al., 1990, Science 249:1438-1441; Weinmaster et al., 1991, Development 113:199-205; Weinmaster et al., 1992, Development 116:931-941; Kopan and Weintraub, 1993, J. Cell Biol. 121:631-641; Ellisen et al., 1991, Cell 66:649-661; Stifani et al., 1992, Nature Genetics 2:119-127; Franco del Amo et al., 1993, Genomics 15:259-264), but does not fall within a conserved position in the consensus sequence compiled for ANK repeats in general (Lux et al., 1990, Nature 344:36-42; Breeden and Nasmyth, 1987, Nature 329:651-654; Michaely and Bennett, 1992, Trends Cell Biol. 2:127-129; Blank et al., 1992, Trends Biochem. Sci. 17:135-140; Robbins et al., 1992, J. Virol. 66:2594-2599).

Interestingly, the su42c mutation, which is the first mutation known to affect the ANK region of Notch, confers upon adult flies a subset of *deltex*-like mutant phenotypes (not shown). These include outstretched wings and variably-fused ocelli (Gorman and Girton, 1992, Genetics 131:99-112; Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677) which are not displayed by the parental Ax^{9B2} mutant. Thus, both the genetic behavior of this mutation as well as its position within the ANK domain implicate this region in Notch/deltex interactions. Biochemical studies should provide insights into whether this mutation alters the affinity of deltex binding and/or interferes with requisite protein conformational changes.

Fig. 11 Methods: Genomic DNA was obtained by PCR and subcloned into Bluescript KS- (Stratagene). Several clones of at least two different PCR reactions were sequenced (both strands) by the dideoxy chain termination method (Sanger et al., 1977, Proc. Natl. Acad. Sci. U.S.A. 74:5463-5467) and covered nucleotides 2850 to 8880 (numbering based on Wharton et al., 1985, Cell 43:567-581). Eleven base-pair changes were discovered, three of which predicted amino acid substitutions. Of these, one at position 3584 corresponded to the previously described Ax^{9B2} mutation (Kelly et

al., 1987, Cell 51:539-548; Hartley et al., 1987, EMBO J. 6:3407-3417) and another at 7510 coincided with a formerly described strain polymorphism (Kidd et al., 1986, Mol. Cell. Biol. 6:3094-3108). A third base-pair change at position 6920 was taken to be the *su42c* mutation and was confirmed by direct sequencing of the PCR product.

7.6. DISCUSSION

We present three independent lines of evidence, the S2 cell assay, *in vivo* co-localization data, and the yeast 'interaction trap' assay, to demonstrate a specific interaction between deltex and the ANK repeats of the Notch intracellular domain. This reveals deltex to be the first cytoplasmic component of a postulated Notch signal transducing complex that also includes Delta and Serrate.

Although we have demonstrated that the ANK repeats are both necessary and sufficient to promote deltex binding, other regions of the intracellular domain may influence the Notch/deltex protein complex. This possibility is raised by the lethal interaction that results when *deltex* mutants also contain the *Notch* mutation *notchoid*^{l}(*nd*^{l}), which is associated with missense mutations near the C terminus (Xu et al., 1990, Genes Dev. 4:464-475). Such influence would be reminiscent of that which occurs in the binding of cactus and dorsal, which are *Drosophila* counterparts of Iκ B and NF-κ B, respectively. Although the ANK repeats of cactus bind to the centrally located Rel homology domain of dorsal, deletion analyses indicate that a region near the carboxy terminus of dorsal also is necessary to keep dorsal sequestered in the cytoplasm when it is bound to cactus (Kidd, 1992, Cell 71:623-635; Robbins et al., 1992, J. Virol. 66:2594-2599; Rushlow et al., 1989, Cell 59:1165-1177; Isoda et al., 1992, Genes Dev. 6:619-630; Norris and Manley, 1992, Genes Dev. 6:1657-1667).

Models analogous to those which have emerged from studies of Iκ B and NF-κ B have been proposed recently to explain the activity of Notch family members (Kidd, 1992, Cell 71:623-635; LaMarco et al., 1991, Science

253:789-792; Kodoyianni et al., 1992, Molec. Biol. Cell 3:1199-1213): Notch may represent a novel Iκ B-like protein that, in response to an external stimulus, causes the dissociation of an ANK repeat-bound transcription factor, which is then transported into the nucleus. However, we find no evidence to support the possibility that deltex may be such a factor. In S2 cells expressing either the ECN or Δ cdc10 deletion constructs (Fig. 8h, i) or in embryos and imaginal tissues over-expressing the deltex protein (see Section 6 and data not shown), we see no nuclear accumulation of deltex at the level of resolution provided by immunofluorescent microscopy (not shown). Truncations of Notch that delete the extracellular and transmembrane regions result in the production of an intracellular domain (N^{sucl}); that localizes within the nucleus (Fortini et al, Nature, in press; Struhl et al., 1993, Cell 74:331-345). Co-expression of deltex with N^{sucl} in S2 cells did not prevent N^{sucl} from translocating into the nucleus and deltex remained in the cytoplasm (not shown).

Molecular analyses have shown that the *Abruptex* alleles of *Notch* represent single amino acid substitutions clustering within six adjacent EGF-like repeats (Kelly et al., 1987, Cell 51:539-548; Hartley et al., 1987, EMBO 6:3407-3417). The phenotype produced by these gain-of-function alleles results in the differentiation of greater numbers of epidermal cells at the expense of neural cells (Heitzler and Simpson, 1993, Development 117:1113-1123) (so-called 'anti-neurogenic' phenotype (Palka et al., 1990, Development 109:167-175)), and contrasts with the opposite phenotype produced by loss-of-function *Notch* mutations. The underlying basis for the dominant phenotype produced by the *Abruptex* mutations is uncertain, although the phenotype is mimicked by truncated forms of Notch (Rebay et al., 1993, Cell 74:319-329; Struhl et al., 1993, Cell 74:331-345), suggesting that this is an activated form of the receptor. On the basis of genetic mosaic studies, Heitzler and Simpson (Heitzler and Simpson, 1993, Development 117:1113-1123) have suggested that Abruptex molecules may have an increased affinity for Delta, but do not constitute constitutively active forms of the receptor, as evidenced by their dependency upon the Delta ligand.

The mechanism by which certain heteroallelic combinations of *Abruptex* alleles 'negatively complement' to result in pupal lethality is uncertain. The conventional interpretation of this phenomenon is that Notch polypeptides interact with one another either as dimers or as part of multiprotein complexes (Foster, 1975, Genetics 81:99-120; Kelly et al., 1987, Cell 51:539-548), a notion that has precedents in a number of other receptor proteins (Ullrich and Schlessinger, 1990, Cell 61:203-212). Pupae of the negatively complementing genotype exhibit a severe loss of sensory bristles compared with *Abruptex* homozygotes (Foster, 1975, Genetics 81:99-120), which are fully viable. This would seem to suggest an even greater gain-of-function activity or 'antineurogenic' effect on the part of the negatively complementing alleles, the consequence of which is inviability. Reducing the gene dosage of either *Delta*, *mastermind*, or *deltex* suppresses both this lethality (Xu et al., 1990, Genes Dev. 4:464-475; Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677) and the sensory bristle loss. In the case of *Delta* and *mastermind*, both of which are neurogenic genes, restoration of viability and sensory bristles may result from a countervailing shift in the developmental equilibrium towards neural differentiation.

In light of our demonstration of a physical Notch/deltex interaction, the finding that lowering the gene dosage of *deltex* also attenuates the apparent 'hyperactivity' of the negatively complementing genotypes implies that deltex normally functions as a positive regulator of Notch activity. This notion is supported by other genetic evidence, namely, the lethal interactions that result from *deltex* and nd^1 (a hypomorphic allele) double mutants and from *deltex* mutants bearing only one wild-type copy of *Notch* (Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677). Also consistent with this idea is the observation that overexpression of deltex in wild-type flies reveals no apparent phenotypic consequences (see Section 6).

The fact that the deltex protein is of unique sequence (see Section 6) raises the possibility that deltex may define a new class of proteins implicated in cell signaling events. Given that the ANK repeats constitute the most

conserved (~70% identity) portion among the various *Notch* homologs (Stifani et al., 1992, Nature Genetics 2:119-127) and that deltex interacts with these repeats, we expect that deltex cognates will exist in higher eukaryotes and that these may function through similar biochemical modes of action.

8. EXAMPLE: PORTIONS OF DELTEX WHICH MEDIATE HETEROTYPIC AND HOMOTYPIC BINDING

Using the interaction trap system as our assay (see Section 7.4) we systematically examined, by deletion analysis, the domains of Notch and deltex which are responsible for protein-protein interactions. Both deltex-deltex as well as deltex-Notch interactions were detected. Deletion constructs encoding various fragments (described below) of *Drosophila* deltex, *Drosophila* Notch and human Notch were expressed as fusion constructs (LexA or ACT fusions), and assayed.

The sequences of the fragments A-D (SEQ ID NOS:5, 6, 7, and 8, respectively) of *Drosophila* deltex which were expressed are shown in Fig. 14.

Figure 13 summarizes the deltex-deltex interactions we have detected. Fragment A interacts with Fragment A (homotypic interactions). Fragment B interacts with Fragment B (homotypic interactions). Fragment C interacts with Fragment C (homotypic interactions). In addition, we detected interactions between fragments C and B. However, we can only detect the fragment C-B interaction if fragment C is tested as the "bait" (*i.e.*, as the LexA fusion). If Fragment B is the bait, this interaction is not detected. All the other aforesaid interactions occur irrespective of which fragment is used as the bait. Fragment A consists of amino acids 1-303. Fragment B consists of amino acids 306-486. Fragment C consists of amino acids 514-737.

The heterotypic interaction between Notch and deltex is occurred between the ANK repeat region of Notch and fragment D of deltex (which is part of fragment A and includes amino acids 1-204). *Drosophila* Notch ANK repeats as well as the ANK repeats of both human Notch proteins (encoded by TAN-1 and hN) were tested in this interaction assay and showed positive binding to fragment D. The following fragments containing the ANK repeat region were used: *Drosophila* Notch amino acids: 1889-2076 (numbering per Wharton et al.,

1985, Cell 43:567-581; see Fig. 17); Human Notch TAN-1 amino acids: 1826-2146 (see Fig. 17); Human Notch hN amino acids: 1772-2093 (see Fig. 17). All displayed interactions with fragment D. Figure 15 summarizes schematically this interaction.

9. EXAMPLE: DELTEX CONTAINS A PUTATIVE SH3-BINDING DOMAIN

SH2 and SH3 domains are conserved protein modules so named based on their homology to the oncogene Src (Src Homology). These motifs have been implicated in mediating protein-protein interactions in a number of signal transduction pathways (reviewed in Cell 71:359-362;Science 252:668-674; Trends Cell Biol. 3:8-13; FEBS 307:55-61). Recently, a complementary motif that binds to the SH3 domain has been identified and called simply an 'SH3-binding domain' ("SH3-BD") (Science 259:1157-1161). The core binding region of SH3-BD is proline-rich and approximately ten residues in length. As shown in Figure 16, this motif, as defined from a mouse protein that experimentally bound an SH3 domain, is shown aligned to two regions (separated by 58 residues) of deltex that may represent *Drosophila* versions of this motif. These regions are located centrally in the deltex protein, approximately 280 residues C-terminal to the region of deltex that has been shown to bind to the ANK repeats of Notch. For reference, regions of selected *Drosophila* proteins, which may also contain SH3-BD, are shown. The *Son of sevenless* (*Sos*) gene, in particular, is worth noting. The encoded protein, a putative guanine nucleotide exchange factor (GNEF), has been shown to bind to an 'adaptor' protein (drk) containing only SH2 and SH3 modules, although the actual residues that mediate binding have not been accurately defined (Cell 73:169-177 and 179-191).

There are currently only six SH3-containing proteins identified in *Drosophila*, any one of which may be a direct binding partner of deltex, and thus an indirect partner of Notch. These include the proteins encoded by the genes *drk*, *src1*, *src2*, *abl*, *spectrin*, and *dlg*. With the exception of spectrin, all encode signalling molecules. Spectrin encodes a protein associated with the cytoskeleton.

Thus deltex may 'tie-in' Notch to already identified signalling components or to the cytoskeleton.

10. DEPOSIT OF MICROORGANISMS

Plasmid pCaSpeR hs-dx containing a cDNA insert encoding a full-length *Drosophila melanogaster* deltex was deposited on January 20, 1994 with the American Type Culture Collection, 1201 Parklawn Drive, Rockville, Maryland 20852, under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedures and assigned accession no. 75640.

The present invention is not to be limited in scope by the microorganism deposited or the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

International Application No: PCT/ /

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 95, lines 1-20 of the description '

A. IDENTIFICATION OF DEPOSIT '

Further deposits are identified on an additional sheet '

Name of depositary institution '

American Type Culture Collection

Address of depositary institution (including postal code and country) '

12301 Parklawn Drive
Rockville, MD 20852
US

Date of deposit ' January 20, 1994 Accession Number ' 75640

B. ADDITIONAL INDICATIONS ' (leave blank if not applicable). This information is continued on a separate attached sheet

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE ' (if the indications are not all designated States)

D. SEPARATE FURNISHING OF INDICATIONS ' (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later ' (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☑ This sheet was received with the International application when filed (to be checked by the receiving Office)

Elnora Y. Rivera
PCT International Division
(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau "

was

(Authorized Officer)

Form PCT/RO/134 (January 1981)

WHAT IS CLAIMED IS:

1. A substantially purified deltex protein.

2. The protein of claim 1 having the amino acid sequence depicted in Figure 12 (SEQ ID NO:4).

3. The protein of claim 1 which is a human protein.

4. The protein of claim 1 which is a *Drosophila* protein.

5. A substantially purified protein comprising a fragment of a deltex protein, said fragment consisting of at least 10 continuous amino acids of the deltex protein.

6. A substantially purified protein comprising a fragment of a deltex protein, said fragment consisting of at least 20 continuous amino acids of the deltex protein.

7. A fragment of a deltex protein consisting of at least 10 continuous amino acids of a deltex protein, which displays one or more functional activities associated with a full-length deltex protein.

8. The fragment of claim 7 which consists of at least 20 continuous amino acids of the deltex protein.

9. The protein of claim 5 in which the protein is able to be bound by an antibody to a deltex protein.

10. The fragment of claim 7 which is able to be bound by an antibody to a deltex protein.

11. A substantially purified protein comprising a fragment of a deltex protein, which fragment comprises a Glu-rich cluster of the deltex protein.

12. A substantially purified protein comprising a fragment of a deltex protein, which fragment binds to a Notch protein or to a molecule comprising the cdc10/SW16/ankyrin repeats of a Notch protein.

13. A substantially purified protein comprising a fragment of a first deltex protein, which fragment binds to a second deltex protein or to a molecule comprising a fragment of a second deltex protein.

14. A substantially purified protein comprising a fragment of a deltex protein, which fragment comprises a SH3 binding domain of the deltex protein.

15. A chimeric protein comprising a functionally active fragment of a deltex protein joined via a peptide bond to an amino acid sequence of a protein other than a deltex protein.

16. The protein of claim 15 in which the fragment binds to a Notch protein or to a molecule comprising the cdc10/SW16/ankyrin repeats of a Notch protein.

17. The protein of claim 15 in which the fragment comprises an SH3 binding domain.

18. A derivative or analog of the protein of claim 1, which is characterized by the ability to be bound by antibody to the protein of claim 1.

19. A peptide having an amino acid sequence in the range of 10-35 amino acids, said sequence being a portion of a deltex protein sequence.

20. A derivative or analog of the protein of claim 1, which is able to display one or more functional activities of the protein of claim 1.

21. An antibody which binds to a deltex protein.

22. The antibody of claim 21 which binds to a *Drosophila* deltex protein.

23. The antibody of claim 21 which binds to a human deltex protein.

24. The antibody of claim 21 which is monoclonal.

25. The antibody of claim 21 which is polyclonal.

26. A fragment or derivative of the antibody of claim 24 containing the binding domain of the antibody.

27. A substantially purified nucleic acid encoding a deltex protein.

28. The nucleic acid of claim 27 which lacks introns.

29. The nucleic acid of claim 27 which encodes a protein having the amino acid sequence depicted in Figure 12 (SEQ ID NO:4).

30. The nucleic acid of claim 28 which comprises the coding region of the nucleotide sequence depicted in Figure 12 (part of SEQ ID NO:3).

31. The nucleic acid of claim 27 which encodes a *Drosophila* deltex protein.

32. The nucleic acid of claim 27 which encodes a human deltex protein.

33. The nucleic acid of claim 28 which encodes a protein comprising the amino acid sequence depicted in Figure 12 (SEQ ID NO:4).

34. A substantially purified first nucleic acid which is hybridizable to a second nucleic acid under conditions of low stringency, said second nucleic acid comprising the nucleotide sequence depicted in Figure 12 (SEQ ID NO:3).

35. A substantially purified first nucleic acid which is hybridizable to a second nucleic acid under conditions of high stringency, said second nucleic acid comprising the nucleotide sequence depicted in Figure 12 (SEQ ID NO:3).

36. A substantially purified first nucleic acid which is hybridizable to a second nucleic acid under conditions of low stringency, said second nucleic acid encoding a protein comprising the amino acid sequence depicted in Figure 12 (SEQ ID NO:4).

37. A substantially purified first nucleic acid which is hybridizable to a second nucleic acid under conditions of high stringency, said second nucleic acid encoding a protein comprising the amino acid sequence depicted in Figure 12 (SEQ ID NO:4).

38. A substantially purified first nucleic acid which is hybridizable to a second nucleic acid under conditions of low stringency, said second nucleic acid comprising the sequence of fragment D as depicted in Figure 14 (SEQ ID NO:8).

39. A substantially purified first nucleic acid which is hybridizable to a second nucleic acid under conditions of high stringency, said second nucleic acid comprising the sequence of fragment D as depicted in Figure 14 (SEQ ID NO:8).

40. A substantially purified first nucleic acid which is hybridizable to a second nucleic acid under conditions of low stringency, said second nucleic acid comprising the sequence of fragment A as depicted in Figure 14 (SEQ ID NO:5).

41. A substantially purified first nucleic acid which is hybridizable to a second nucleic acid under conditions of high stringency, said second nucleic acid comprising the sequence of fragment A as depicted in Figure 14 (SEQ ID NO:5).

42. A substantially purified first nucleic acid which is hybridizable to a second nucleic acid under conditions of low stringency, said second nucleic acid comprising the sequence of fragment B as depicted in Figure 14 (SEQ ID NO:6).

43. A substantially purified first nucleic acid which is hybridizable to a second nucleic acid under conditions of high stringency, said second nucleic acid comprising the sequence of fragment B as depicted in Figure 14 (SEQ ID NO:6).

44. A substantially purified first nucleic acid which is hybridizable to a second nucleic acid under conditions of low stringency, said second nucleic acid comprising the sequence of fragment C as depicted in Figure 14 (SEQ ID NO:7).

45. A substantially purified first nucleic acid which is hybridizable to a second nucleic acid under conditions of high stringency, said second nucleic acid comprising the sequence of fragment C as depicted in Figure 14 (SEQ ID NO:7).

46. A substantially purified nucleic acid encoding the protein of claim 5.

47. A substantially purified nucleic acid encoding the protein of claim 12.

48. A substantially purified nucleic acid encoding the protein of claim 13.

49. A substantially purified nucleic acid encoding the protein of claim 14.

50. A nucleic acid encoding the chimeric protein of claim 15.

51. The nucleic acid of claim 27 as contained in plasmid pCaSpeR hs-dx as deposited with the ATCC and assigned accession number 75640.

52. A nucleic acid vector comprising the nucleic acid of claim 27.

53. A nucleic acid vector comprising the nucleic acid of claim 28.

54. A recombinant cell containing the nucleic acid vector of claim 52.

55. A recombinant cell containing the nucleic acid vector of claim 53.

56. A method for producing a deltex protein comprising growing the recombinant cell of claim 54, such that the deltex protein is expressed by the cell; and recovering the expressed deltex protein.

57. A method for producing a protein comprising growing a cell containing a recombinant nucleic acid comprising the nucleic acid of claim 36, such that the protein is expressed by the cell; and recovering the expressed protein.

58. A pharmaceutical composition comprising a therapeutically effective amount of a deltex protein; and a pharmaceutically acceptable carrier.

59. The composition of claim 58 in which the deltex protein is a human deltex protein.

60. A pharmaceutical composition comprising a therapeutically effective amount of the protein of claim 7; and a pharmaceutically acceptable carrier.

61. A pharmaceutical composition comprising a therapeutically effective amount of the protein of claim 12; and a pharmaceutically acceptable carrier.

62. A pharmaceutical composition comprising a therapeutically effective amount of the protein of claim 13; and a pharmaceutically acceptable carrier.

63. A pharmaceutical composition comprising a therapeutically effective amount of the protein of claim 14; and a pharmaceutically acceptable carrier.

64. A pharmaceutical composition comprising a therapeutically effective amount of the protein of claim 15; and a pharmaceutically acceptable carrier.

65. A pharmaceutical composition comprising a therapeutically effective amount of a derivative or analog of a deltex protein, which derivative or analog is characterized by the ability to bind to a Notch protein or to a molecule comprising the cdc10/SW16/ankyrin repeats of a Notch protein.

66. A pharmaceutical composition comprising a therapeutically effective amount of a nucleic acid encoding a deltex protein; and a pharmaceutically acceptable carrier.

67. A pharmaceutical composition comprising a therapeutically effective amount of the nucleic acid of claim 34; and a pharmaceutically acceptable carrier.

68. A pharmaceutical composition comprising a therapeutically effective amount of a nucleic acid of claim 40; and a pharmaceutically acceptable carrier.

69. A pharmaceutical composition comprising a therapeutically effective amount of an antibody which binds to a deltex protein; and a pharmaceutically acceptable carrier.

70. A pharmaceutical composition comprising a therapeutically effective amount of a fragment or derivative of an antibody to a deltex protein

containing the binding domain of the antibody; and a pharmaceutically acceptable carrier.

71. A method of treating or preventing a disease or disorder in a subject comprising administering to a subject in need of such treatment or prevention a therapeutically effective amount of a molecule which antagonizes the function of a deltex protein.

72. The method according to claim 71 in which the disease or disorder is a malignancy characterized by increased Notch activity or increased expression of a Notch protein or of a Notch derivative capable of being bound by an anti-Notch antibody, relative to said Notch activity or expression in an analogous non-malignant sample.

73. The method according to claim 71 in which the disease or disorder is cervical cancer.

74. The method according to claim 71 in which the disease or disorder is breast cancer.

75. The method according to claim 71 in which the disease or disorder is colon cancer.

76. The method according to claim 71 in which the malignancy is selected from the group consisting of melanoma, seminoma, and lung cancer.

77. The method according to claim 72 in which the subject is a human.

78. The method according to claim 71 in which the molecule is an antibody to deltex or a derivative of said antibody containing the binding domain thereof.

79. The method according to claim 71 in which the molecule is a protein comprising a portion of a deltex protein capable of binding to a Notch protein or to a second molecule comprising the cdc10/SW16/ankyrin repeats of a Notch protein.

80. The method according to claim 71 in which the molecule is a protein comprising the SH3 binding domain of a deltex protein.

81. The method according to claim 71 in which the molecule is an oligonucleotide which (a) consists of at least six nucleotides; (b) comprises a sequence complementary to at least a portion of an RNA transcript of a *deltex* gene; and (c) is hybridizable to the RNA transcript.

82. A method of treating or preventing a disease or disorder in a subject in need of such treatment or prevention comprising administering to the subject a therapeutically effective amount of a molecule which promotes the function of a deltex protein.

83. A method of treating or preventing a malignancy in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of a deltex protein.

84. A method of treating or preventing a malignancy in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of the nucleic acid of claim 27.

85. A method of treating or preventing a malignancy in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of the antibody of claim 21.

86. A method for treating a patient with a tumor, of a tumor type characterized by expression of a *Notch* or *deltex* gene, comprising administering to the patient an effective amount of an oligonucleotide, which oligonucleotide (a) consists of at least six nucleotides; (b) comprises a sequence complementary to at least a portion of an RNA transcript of the *deltex* gene; and (c) is hybridizable to the RNA transcript.

87. An isolated oligonucleotide consisting of at least six nucleotides, and comprising a sequence complementary to at least a portion of an RNA transcript of a *deltex* gene, which oligonucleotide is hybridizable to the RNA transcript.

88. A pharmaceutical composition comprising the oligonucleotide of claim 87; and a pharmaceutically acceptable carrier.

89. A method of inhibiting the expression of a nucleic acid sequence encoding a deltex protein in a cell comprising providing the cell with an effective amount of the oligonucleotide of claim 87.

90. A method of diagnosing a disease or disorder characterized by an aberrant level of Notch-deltex protein binding activity in a patient, comprising measuring the ability of a Notch protein in a sample derived from the patient to bind to a deltex protein, in which an increase or decrease in the ability of the Notch protein to bind to the deltex protein, relative to the ability found in an analogous sample from a normal individual, indicates the presence of the disease or disorder in the patient.

91. A method of diagnosing a disease or disorder characterized by an aberrant level of Notch-deltex protein binding activity in a patient, comprising measuring the ability of a deltex protein in a sample derived from the patient to bind to a Notch protein, in which an increase or decrease in the ability of the deltex protein to bind to the Notch protein, relative to the ability found in an analogous sample from a normal individual, indicates the presence of the disease or disorder in the patient.

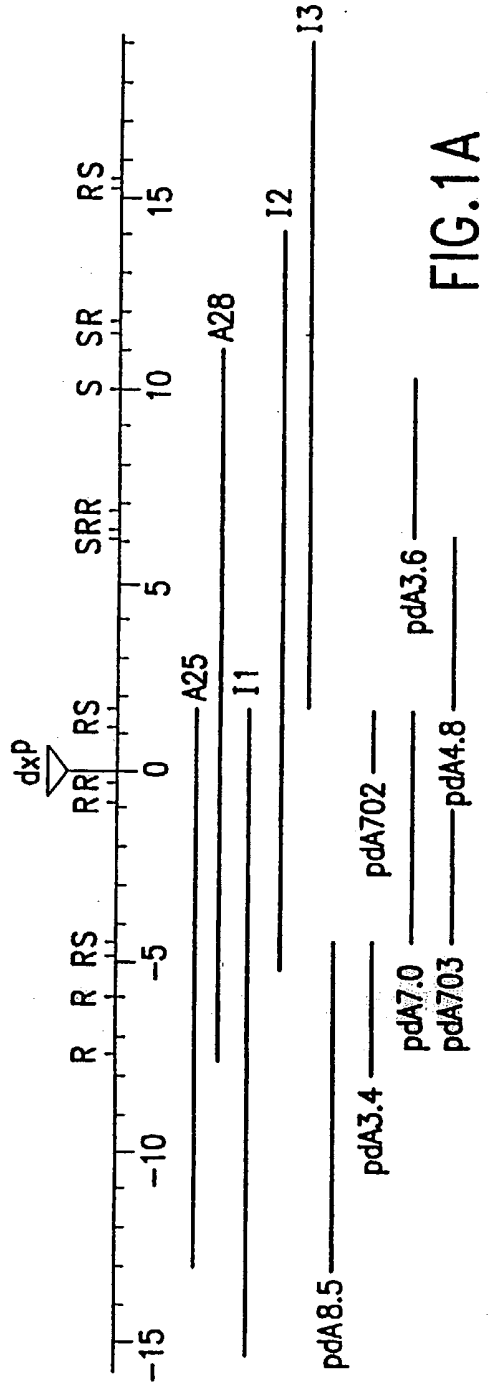
dxP
R R RS RS SRR S SR RS
−15 −10 −5 0 5 10 15
A25
A28
I1
I2
I3
pdA8.5
pdA3.4
pdA702
pdA7.0
pdA703
pdA4.8
pdA3.6

FIG.1A

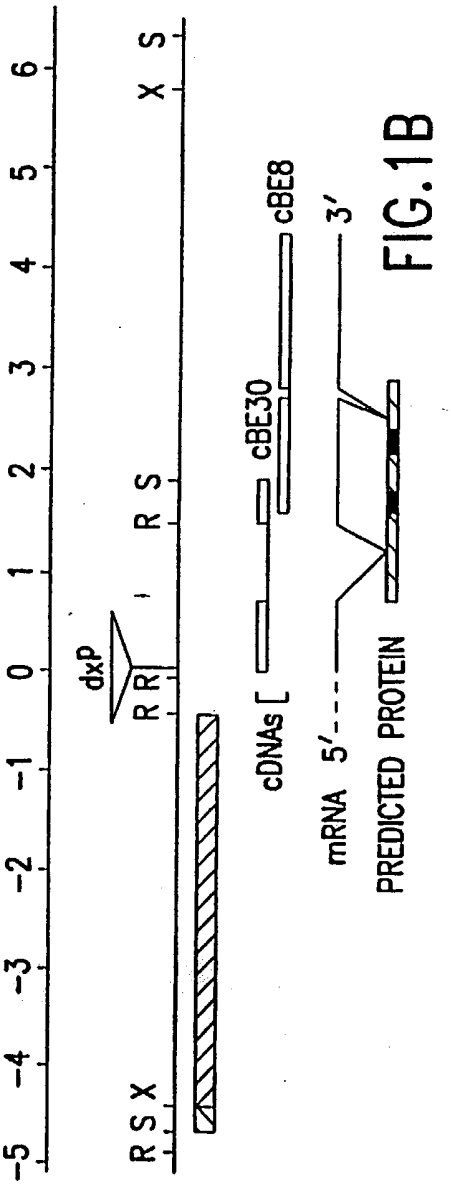
−5 −4 −3 −2 −1 0 1 2 3 4 5 6
dxP
R R
R S
X S
R S X
cDNAs [
cBE30
cBE8
mRNA 5′
3′
PREDICTED PROTEIN

FIG.1B

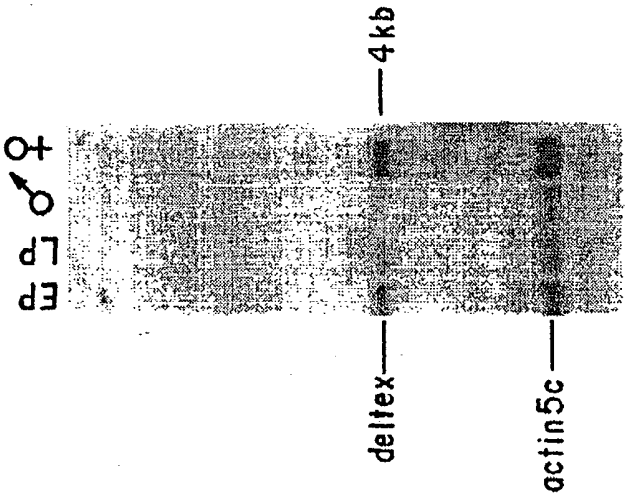
4kb
EP
LP
♀
♂
deltex
actin5c

FIG. 2B

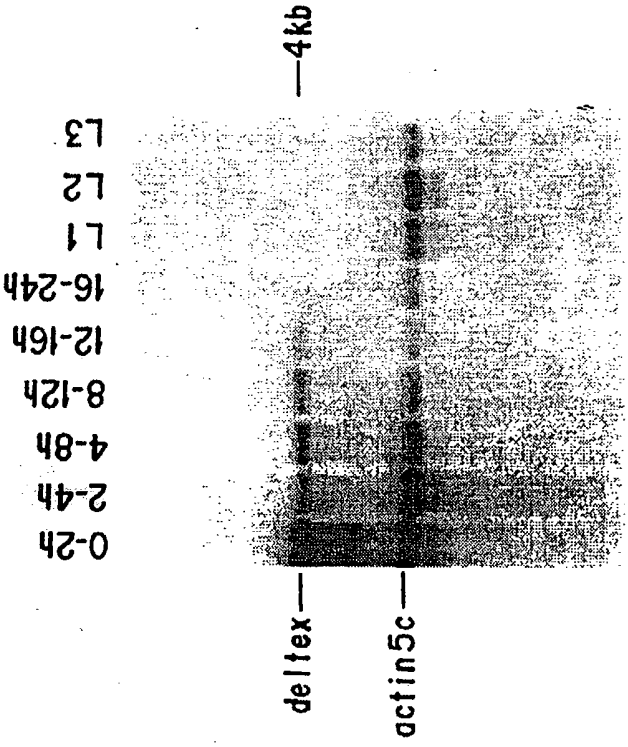
4kb
L3
L2
L1
16-24h
12-16h
8-12h
4-8h
2-4h
0-2h
deltex
actin5c

FIG. 2A


acatacaccacaaatgtggtcgttacagcaaagacatgctcaaattatgaatttcttaaaagtgaccgatttgtattaccgaaaatcttgaaaagaaatactaaatagaa
agaaacttggaagcagccagtgttggaggctgcgttgcttaagaaaagtagcttaaacttgccaaagtaaatacaaaaatattgaaattaatatcaagtaaaatcattgcaagttcttat
ttttttcatttcaatatagaattcaattacacatatattaattgattaattaacaagctaaaaatagtatctgtttatattcaagtgcaagaaaagaaaatacaaaaatattaaacattta
gctagatctagctagcaaccagttatgccaacccctgaaactaccgaatccaactggtggctgcgagatgcagtgcggtattttcatccggccttatttagccatttccgctgttgtctc
1 tcattaaatcgctgtccaacaatacaaaattttctcgcctgcgcttcgcgtAAATGCTAGAAAAACCGTTTTTACCATCAAACGTGAATTCTTAAGCTGCGCCTAAACGAAACCGAGTGACT
81 AAAGAACCAGAACGAAAACTTCGGGAAAATGGAAGCCAGGGAAAATCAGGGATAACTAACGCTGGCAGCGGGTCCACCATTTTTAATTTCTTTGTTTATTTTGTGCCCATCTTCGCGAGC
201 GAGCGAGATAGCGCGACAGCAACAGCAAGAGAGAGCGAGAGAGAGAGTGAGTGAGTGAGAGCTAGTGAAGAGAGCGCAGGAGGAGTTGGATATGGAAATGGGCATGGATATGGCAATGGG
321 CTCACTCCACGGATAACGGATCAACTGCAAGCAATGGCCAGCAGCGCCGGAAGTGCGGCATCCGGATCCGTTGTTCCCGGTGGCGGAGGTAGCGCCGCCTCCAGTTGTGCCACCATGGCC
M A S S A G S A A S G S V V P G G G G S A A S S C A T M A 29
441 CTGTCCACCGCCGGATCCGGTGGGCCGCCCGTGAACCACGCCCACGCCGTCTGCGTGTGGAGTTCGAGTCGCGCGGCAAGTGGCTGCCCTATTCGCCCGGCGGTGTCGCAGCACTTGGAA
L S T A G S G G P P V N H A H A V C V W E F E S R G K W L P Y S P A V S Q H L E 69
561 CGCGCCCACGCCAAGAAACTGACGCGCGTCATGCTGAGCGATGCGGATCCCAGCCTGGAGCAGTACTACGTCAACGTGCGCACAATGACCCAGGAATCGGAGGCGGAAACGCGCTCCGGC
R A H A K K L T R V M L S D A D P S L E Q Y Y V N V R T M T Q E S E A E T R S G 109
681 CTGCTGACCATCGGTGTTCGGCGCATGTTATACGCACCCAGCTCGCCGGCGGGCAAGGGCACCAAGTGGGAGTGGTCGGGCGGCAGTGCCGATAGCAACAACGACTGGCGGCCCTACAAC
L L T I G V R R M L Y A P S S P A G K G T K W E W S G G S A D S N N D W R P Y N 149
801 ATGCACGTCCAGTGCATCATCGAGGACGCCTGGGCGAGGgtgagtgccgtgcagaatcgaatgttttcccattggcagtctagttggaatttgagttgctcaggaatttttttatcggt
M H V Q C I I E D A W A R 162
agaatatatagggaatctttaaagagtgtgggtttaccactgatcctgaagaattggctaaggatgcaccagtctgtgatgtgtccataggtggttaaatagtacatattagccaaattc
cacagttcacatctcagccaaaaaacgtagtagatcgaagaagtgtaccttttgaatttctgatgcttagacaaataaatctgcatttaatttttttaactaaaatcaaattaatttttt

FIG.3A

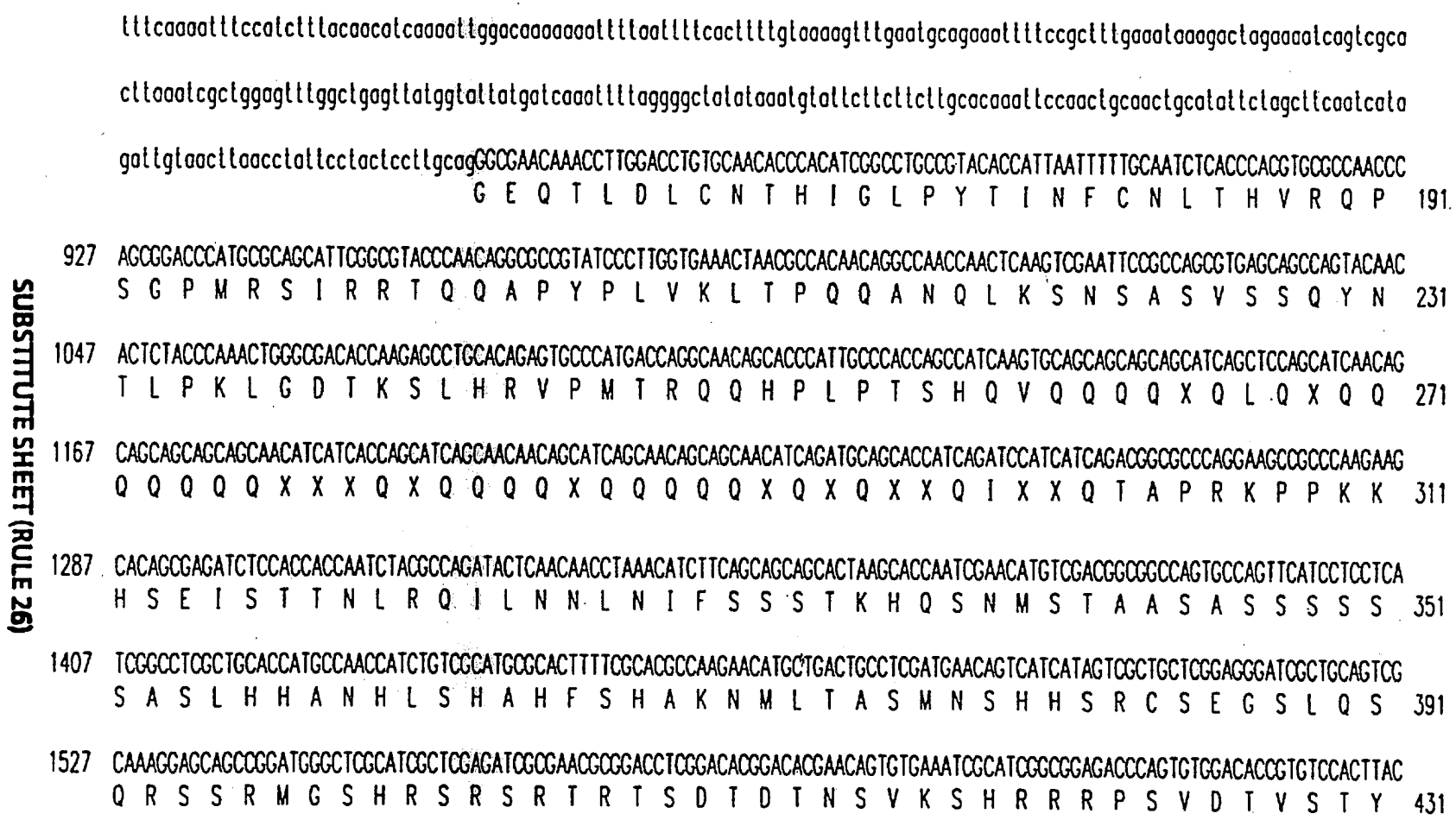
tttcaaaatttccatctttacaacatcaaaattggacaaaaaaattttaattttcacttttgtaaaagtttgaatgcagaaattttccgctttgaaataaagactagaaaatcagtcgca
cttaaatcgctggagtttggctgagttatggtattatgatcaaattttaggggctatataaatgtattcttcttcttgcacaaattccaactgcaactgcatattctagcttcaatcata
gattgtaacttaaccctattcctactccttgcagGGCGAACAAACCTTGGACCTGTGCAACACCCACATCGGCCTGCCGTACACCATTAATTTTTGCAATCTCACCCACGTGCGCCAACCC
G E Q T L D L C N T H I G L P Y T I N F C N L T H V R Q P 191
927 AGCGGACCCATGCGCAGCATTCGGCGTACCCAACAGGCGCCGTATCCCTTGGTGAAACTAACCCCACAACAGGCCAACCAACTCAAGTCGAATTCCGCCAGCGTGAGCAGCCAGTACAAC
S G P M R S I R R T Q Q A P Y P L V K L T P Q Q A N Q L K S N S A S V S S Q Y N 231
1047 ACTCTACCCAAACTGGGCGACACCAAGAGCCTGCACAGAGTGCCCATGACCAGGCAACAGCACCCATTGCCCACCAGCCATCAAGTGCAGCAGCAGCAGCATCAGCTCCAGCATCAACAG
T L P K L G D T K S L H R V P M T R Q Q H P L P T S H Q V Q Q Q Q X Q L Q X Q Q 271
1167 CAGCAGCAGCAGCAACATCATCACCAGCATCAGCAACAACAGCATCAGCAACAGCAGCAACATCAGATGCAGCACCATCAGATCCATCATCAGACGGCGCCCAGGAAGCCGCCCAAGAAG
Q Q Q Q Q X X X Q X Q Q Q Q X Q Q Q Q Q X Q X Q X X Q I X X Q T A P R K P P K K 311
1287 CACAGCGAGATCTCCACCACCAATCTACGCCAGATACTCAACAACCTAAACATCTTCAGCAGCAGCACTAAGCACCAATCGAACATGTCGACGGCGGCCAGTGCCAGTTCATCCTCCTCA
H S E I S T T N L R Q I L N N L N I F S S S T K H Q S N M S T A A S A S S S S S 351
1407 TCGGCCTCGCTGCACCATGCCAACCATCTGTCGCATGCGCACTTTTCGCACGCCAAGAACATGCTGACTGCCTCGATGAACAGTCATCATAGTCGCTGCTCGGAGGGATCGCTGCAGTCG
S A S L H H A N H L S H A H F S H A K N M L T A S M N S H H S R C S E G S L Q S 391
1527 CAAAGGAGCAGCCGGATGGGCTCGCATCGCTCGAGATCGCGAACGCGGACCTCGGACACGGACACGAACAGTGTGAAATCGCATCGGCGGAGACCCAGTGTGGACACCGTGTCCACTTAC
Q R S S R M G S H R S R S R T R T S D T D T N S V K S H R R R P S V D T V S T Y 431

FIG.3B

```
1647  CTCAGCCACGAGAGCAAGGAGAGCCTGCGCAGCAGGAACTTTGCCATTTCGGTCAATGATCTGCTGGACTGCTCGCTTGGCAGCGATGAAGTTTTTGTGCCCTCCGTGCCGCCATCGTCG
      L  S  H  E  S  K  E  S  L  R  S  R  N  F  A  I  S  V  N  D  L  L  D  C  S  L  G  S  D  E  V  F  V  P  S  V  P  P  S  S  471

1767  CTGGGCGAAAGGGCGCCGGTGCCGCCGCCATTACCACTGCATCCGCGACAGCAACAGCAGCAGCAACAACAGCAGCAACAGCTGCAGATGCAACAGCAGCAACAGGCGCAGCAGCAGCAG
      L  G  E  R  A  P  V  P  P  P  L  P  L  H  P  R  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  L  Q  X  Q  Q  Q  Q  Q  X  Q  Q  Q  Q  511

1887  CAGCAATCAATCGCCGGTTCGATTGTGGGCGTGGACCCGGCCAGCGATATGATATCGCGTTTTGTCAAGGTGGTGGAGCCACCGCTGTGGCCCAATGCCCAGCCCTGTCCCATGTGCATG
      Q  Q  S  I  A  G  S  I  V  G  V  D  P  A  S  D  M  I  S  R  F  V  K  V  V  E  P  P  L  W  P  N  A  Q  P  C  P  M  C  M  551

2007  GAGGAGCTGGTGCACTCCGCCCAGAATCCGGCCATTTCGCTGAGTCGCTGCCAGCATCTCATGCATTTGCAGTGCCTCAATGGGATGATAATTGCCCAGCAAAACGAAATGAACAAGAAC
      E  E  L  V  H  S  A  Q  N  P  A  I  S  L  S  R  C  Q  H  L  M  H  L  Q  C  L  N  G  M  I  I  A  Q  Q  N  E  M  N  K  N  591

2127  CTTTTCATCGAGTGCCCTGTATGCGGCATCGTTTACGCGCAGAAGGTCGGCAATCAGCCCATTGGCAGCATGTCGTGGAGCATAATTAGCAAGAATCTGCCAGGACACGAGGGTCAGAAC
      L  F  I  E  C  P  V  C  G  I  V  Y  G  E  K  V  G  N  Q  P  I  G  S  M  S  W  S  I  I  S  K  N  L  P  G  H  E  G  Q  N  631

2247  ACCATACAGATTGTTTACGAgtaagtgtgaatgtgcctgtggccactgagcaatcaactataatcactcttttcatttgcatggcagCATTGCATCGGGACTGCAGACGGAGGAGCATC
      T  I  Q  I  V  Y  D                                                                                  I  A  S  G  L  Q  T  E  E  H  P  649

2299  CGCATCCAGGTCGTGCCTTCTTCGCCGTGGGATTCCCGCGGATCTGCTACTTGCCGGACTGCCCGCTGGGGCGAAAGGTTTTGCGCTTCCTCAAGATTGCATTCGATCGTCGGCTGCTTT
        H  P  G  R  A  F  F  A  V  G  F  P  R  I  C  Y  L  P  D  C  P  L  G  R  K  V  L  R  F  L  K  I  A  F  D  R  R  L  L  F  689

2419  TCTCGATCGGACGATCGGTGACCACCGGACGCGAGGATGTGGTGATCTGGAACAGTGTGGATCACAAGACGCAGTTCAATATGTTTCCGGATCCCACCTATTTGCAGCGAACCATGCAAC
        S  I  G  R  S  V  T  T  G  R  E  D  V  V  I  W  N  S  V  D  H  K  T  Q  F  N  M  F  P  D  P  T  Y  L  Q  R  T  M  Q  Q  729
```

FIG.3C

2539 AGCTGGTGCACCTGGGCGTGACGGATTAAGGATTAGTTCCCTGTCCCCAAGTAGAACTACCAACCAACCAATCAACCACCCACCCACCGAAGTCCCCTCGATCATTCTCTTCCATTCGTC
L V H L G V T D 737

2659 GTTAAGTTACTTTCTACATAATCTCAGTGTGTGTGCAATCCTCGTTTACTATGATATATTTTTTTTATAGATATATTGTAATAGCGTTCGAGCTGCTCGAACCCTAAAACAACAGCAAAC

2779 CACAATTGCAATTGTAGCTTCCTTTCCGCTCTTCCAATTCGTATTTGTACGCACATACGCAATAAGTTGGCGTACATCATATGTATTAGCTAGTTAGTTAGTTAGTTAGTTGTAGC

2899 TGTAGTTCCCAAGAGAATCTTGACCCAAGACACCTACTAGTATTAGGCATTATCCTGATTCTTGATTCCTGATTCGATTCAAGCCAAGCCAAGCCACGCCATTCGAGTGCAAGCTGTGCC

3019 AAAATCGTAGCGCTCCCGTTTATAGGATATGTATATTGTTGATATAGCTAGCTATAACCATTGCCCATCTCTCCATCTCTCTCGGTTTCGAATTTGTCTCTTTCATCAGATCCATGTGAA

3139 TTTTCTTTATATCGGATTTATATAGGATTAAAATAGTATTTTGAGAGAGGAAATGGAGATGGGTAAATTCGATAGACTTGTCTCACTTGTCTTGGCCATTTAATCTCTTTCATTCAGCGA

3259 ATTTGATGTGATTTTAATTTGAATTATTCATTATTAAACGGAGCATTTAGGAAGCATAGTTGTAACGCAGCCAGATATTCCATTACGCATATACATATACATATACATATACATACATAC

3379 ATAAACATATTTTAACATAGCCCCATAGCCATACGACATAACAATAATTTTTTTTATCGAATCCCTTGCATACATTTGATGAATTGTTGCTTTCATATTGATATCATCGAGCATCGAACG

3499 AACTATCGTATACATCGCCAATATATAGCATATATAGCATATAGTATGTAGAGATCGTACGGACAGCTAGCGGCTACTGACCGCGCCACCATATTTGATATGATATGATATGATTTTACT

3619 AAGTTGTATTTAGCACTGATTAGTTATTAAAGTTCATTTGACGAATATTCCACAACAAATTCCACACCATTTCTGTATGCATATTACGCATATATAATACAGTACATTTATATATAGTTC

3739 AAATAAAGTAACTTTCATTCATGTTCaaattaagtctttctttgggatatttattcactcatgtctaaaaggaaatctttctttttggactttttcacttatgtatgtatgttcgaatg

cgttctttctttgggactttttttctttatgc

FIG.3D

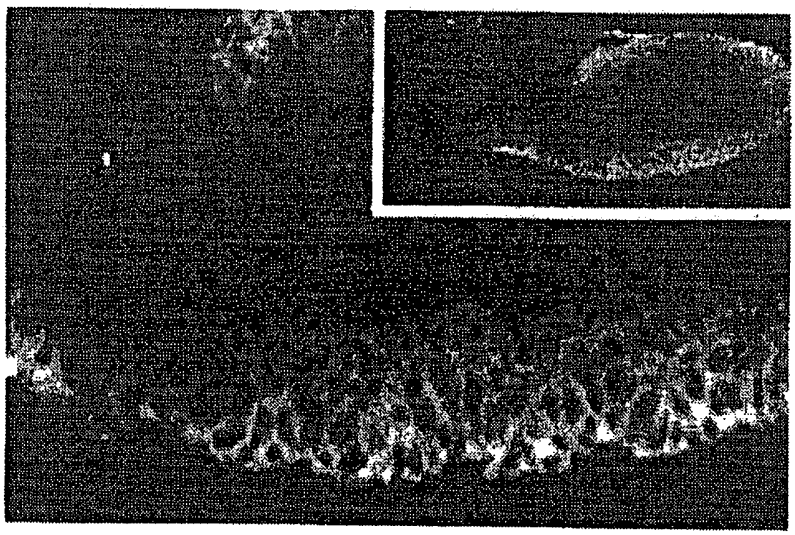

FIG. 4

allele	$Su(dx)^{sp}/+$	$Su(dx)/+$	$Su(dx)^{2}/+$
dx^{ENU}	+++	+++	+++
dx^{SM}	+++	+++	+
dx	++	++	+ -
dx^{p}	+	+	+

FIG.5

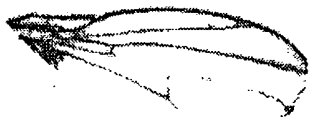

FIG. 6A

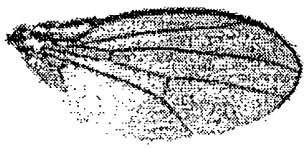

FIG. 6B

FIG. 6C

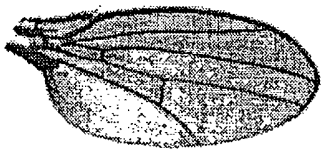

FIG. 6D

FIG. 6E

FIG. 6F

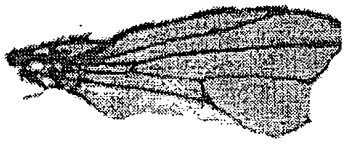

FIG. 7A

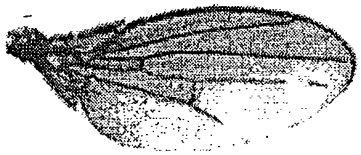

FIG. 7B

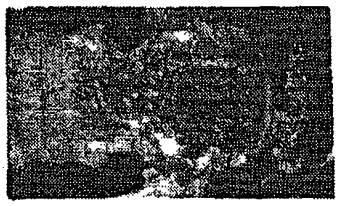

FIG. 7C

FIG. 7D

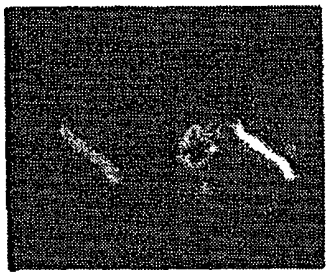

FIG. 8A

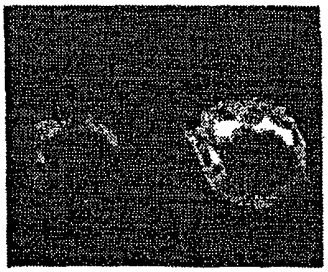

FIG. 8B

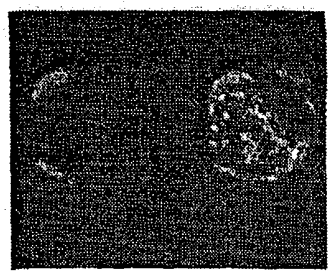

FIG. 8C

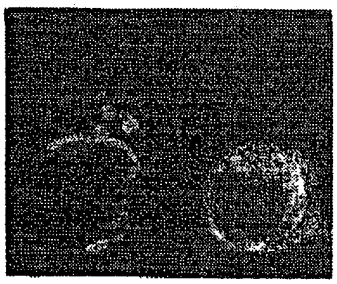

FIG. 8D

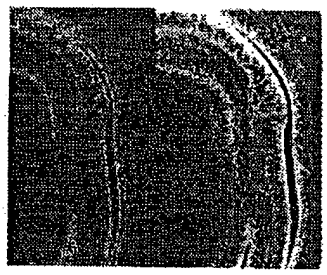

FIG. 8E

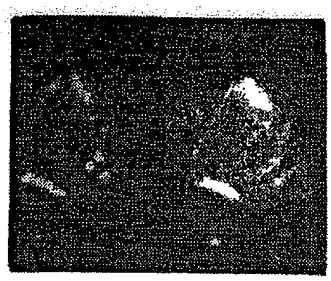

FIG. 8F

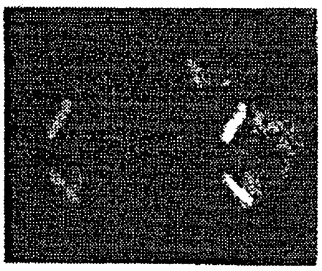

FIG. 8G

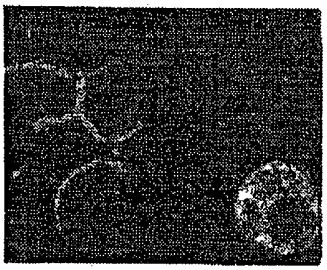

FIG. 8H

FIG. 8I

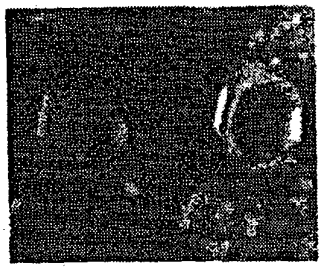

FIG. 8J

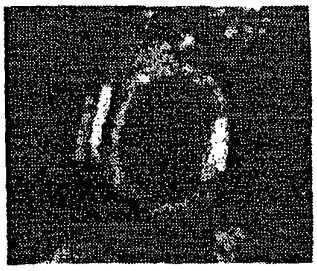

FIG. 8K

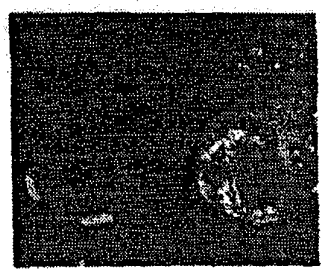

FIG. 8L

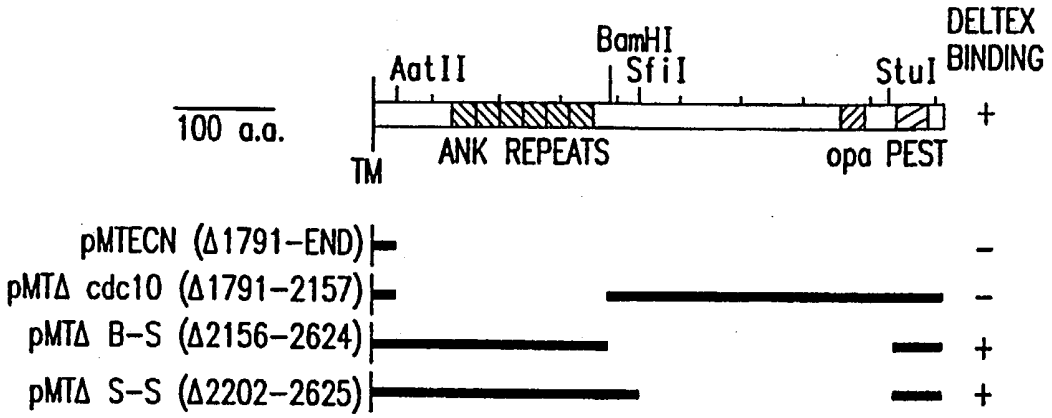

FIG.9A

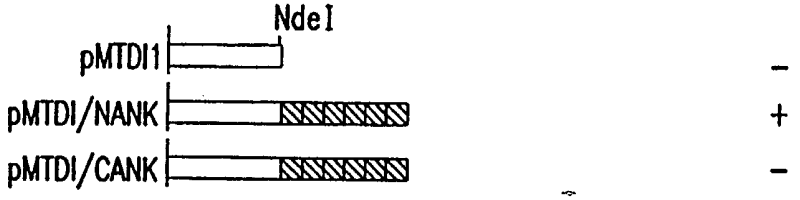

FIG.9B

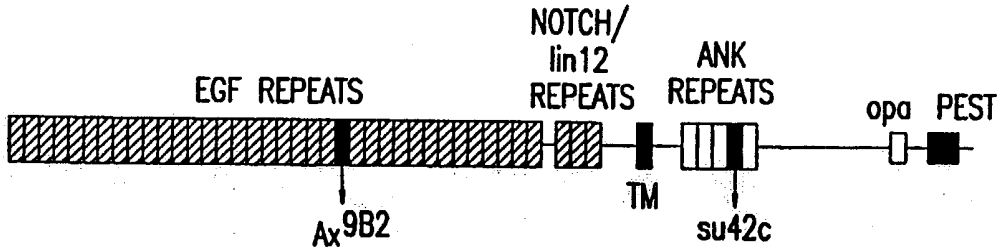

FIG.11A

2044

I N A A D N S G K T A L H W A A A V N N T E A V N I L L M H H A N

V

FIG.11C

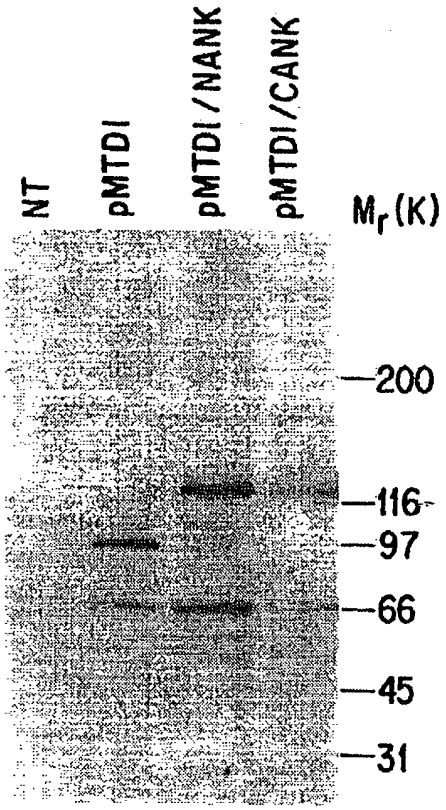

FIG. 9C

LexA DNA binding fusion	Notch ICN1	Notch ICN1	Notch ICN1	bicoid	groucho	LexA alone
acidic activator fusion	deltex	groucho	activator alone	deltex	deltex	deltex

FIG.10

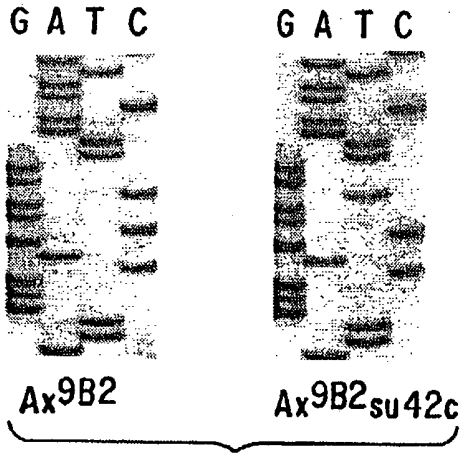

FIG. 11B

```
      10         20         30         40         50         60
   *   *   *   *   *   *   *   *   *   *   *   *
AAATGCTAGA AAAACCGTTT TTACCATCAA ACGTGAATTC TTAAGCTGCG CCTAAACGAA
TTTACGATCT TTTTGGCAAA AATGGTAGTT TGCACTTAAG AATTCGACGC GGATTTGCTT

      70         80         90        100        110        120
   *   *   *   *   *   *   *   *   *   *   *   *
ACCGAGTGAC TAAAGAACCA GAACGAAAAC TTCGGGAAAA TGGAAGCCAG GGAAAATCAG
TGGCTCACTG ATTTCTTGGT CTTGCTTTTG AAGCCCTTTT ACCTTCGGTC CCTTTTAGTC

     130        140        150        160        170        180
   *   *   *   *   *   *   *   *   *   *   *   *
GGATAACTAA CGCTGGCAGC GGGTCCACCA TTTTTAATTT CTTTGTTTAT TTTGTGCCCA
CCTATTGATT GCGACCGTCG CCCAGGTGGT AAAAATTAAA GAAACAAATA AAACACGGGT

     190        200        210        220        230        240
   *   *   *   *   *   *   *   *   *   *   *   *
TCTTCGCGAG CGAGCGAGAT AGCGCGACAG CAACAGCAAG AGAGAGCGAG AGAGAGAGTG
AGAAGCGCTC GCTCGCTCTA TCGCGCTGTC GTTGTCGTTC TCTCTCGCTC TCTCTCTCAC

     250        260        270        280        290        300
   *   *   *   *   *   *   *   *   *   *   *   *
AGTGAGTGAG AGCTAGTGAA GAGAGCGCAG GAGGAGTTGG ATATGGAAAT GGGCATGGAT
TCACTCACTC TCGATCACTT CTCTCGCGTC CTCCTCAACC TATACCTTTA CCCGTACCTA

     310        320        330        340        350        360
   *   *   *   *   *   *   *   *   *   *   *   *
ATGGCAATGG GCTCACTCCA CGGATAACGG ATCAACTGCA AGCAATGGCC AGCAGCGCCG
TACCGTTACC CGAGTGAGGT GCCTATTGCC TAGTTGACGT TCGTTACCGG TCGTCGCGGC
                                              M  A  S  S  A>

     370        380        390        400        410        420
   *   *   *   *   *   *   *   *   *   *   *   *
GAAGTGCGGC ATCCGGATCC GTTGTTCCCG GTGGCGGAGG TAGCGCCGCC TCCAGTTGTG
CTTCACGCCG TAGGCCTAGG CAACAAGGGC CACCGCCTCC ATCGCGGCGG AGGTCAACAC
G  S  A  A   S  G  S   V  V  P   G  G  G  G   S  A  A   S  S  C>

     430        440        450        460        470        480
   *   *   *   *   *   *   *   *   *   *   *   *
CCACCATGGC CCTGTCCACC GCCGGATCCG GTGGGCCGCC CGTGAACCAC GCCCACGCCG
GGTGGTACCG GGACAGGTGG CGGCCTAGGC CACCCGGCGG GCACTTGGTG CGGGTGCGGC
A  T  M  A   L  S  T   A  G  S   G  G  P  P   V  N  H   A  H  A>
```

FIG.12A

```
      490        500        510        520        530        540
    *   *    *   *    *   *    *   *    *   *    *   *    *   *
TCTGCGTGTG GGAGTTCGAG TCGCGCGGCA AGTGGCTGCC CTATTCGCCG GCGGTGTCGC
AGACGCACAC CCTCAAGCTC AGCGCGCCGT TCACCGACGG GATAAGCGGC CGCCACAGCG
V  C  V  W   E  F  E   S  R  G   K  W  L  P   Y  S  P   A  V  S>

      550        560        570        580        590        600
    *   *    *   *    *   *    *   *    *   *    *   *    *   *
AGCACTTGGA ACGCGCCCAC GCCAAGAAAC TGACGCGCGT CATGCTGAGC GATGCGGATC
TCGTGAACCT TGCGCGGGTG CGGTTCTTTG ACTGCGCGCA GTACGACTCG CTACGCCTAG
Q  H  L  E   R  A  H   A  K  K   L  T  R  V   M  L  S   D  A  D>

      610        620        630        640        650        660
    *   *    *   *    *   *    *   *    *   *    *   *    *   *
CCAGCCTGGA GCAGTACTAC GTCAACGTGC GCACAATGAC CCAGGAATCG GAGGCGGAAA
GGTCGGACCT CGTCATGATG CAGTTGCACG CGTGTTACTG GGTCCTTAGC CTCCGCCTTT
P  S  L  E   Q  Y  Y   V  N  V   R  T  M  T   Q  E  S   E  A  E>

      670        680        690        700        710        720
    *   *    *   *    *   *    *   *    *   *    *   *    *   *
CGCGCTCCGG CCTGCTGACC ATCGGTGTTC GGCGCATGTT ATACGCACCC AGCTCGCCGG
GCGCGAGGCC GGACGACTGG TAGCCACAAG CCGCGTACAA TATGCGTGGG TCGAGCGGCC
T  R  S  G   L  L  T   I  G  V   R  R  M  L   Y  A  P   S  S  P>

      730        740        750        760        770        780
    *   *    *   *    *   *    *   *    *   *    *   *    *   *
CGGGCAAGGG CACCAAGTGG GAGTGGTCGG GCGGCAGTGC CGATAGCAAC AACGACTGGC
GCCCGTTCCC GTGGTTCACC CTCACCAGCC CGCCGTCACG GCTATCGTTG TTGCTGACCG
A  G  K  G   T  K  W   E  W  S   G  G  S  A   D  S  N   N  D  W>

      790        800        810        820        830        840
    *   *    *   *    *   *    *   *    *   *    *   *    *   *
GGCCCTACAA CATGCACGTC CAGTGCATCA TCGAGGACGC CTGGGCGAGG GGCGAACAAA
CCGGGATGTT GTACGTGCAG GTCACGTAGT AGCTCCTGCG GACCCGCTCC CCGCTTGTTT
R  P  Y  N   M  H  V   Q  C  I   I  E  D  A   W  A  R   G  E  Q>

      850        860        870        880        890        900
    *   *    *   *    *   *    *   *    *   *    *   *    *   *
CCTTGGACCT GTGCAACACC CACATCGGCC TGCCGTACAC CATTAATTTT TGCAATCTCA
GGAACCTGGA CACGTTGTGG GTGTAGCCGG ACGGCATGTG GTAATTAAAA ACGTTAGAGT
T  L  D  L   C  N  T   H  I  G   L  P  Y  T   I  N  F   C  N  L>
```

FIG.12B

```
      910        920        930        940        950        960
    *   *    *   *    *   *    *   *    *   *    *   *    *   *
CCCACGTGCG CCAACCCAGC GGACCCATGC GCAGCATTCG GCGTACCCAA CAGGCGCCGT
GGGTGCACGC GGTTGGGTCG CCTGGGTACG CGTCGTAAGC CGCATGGGTT GTCCGCGGCA
T  H  V  R   Q  P  S   G  P  M   R  S  I  R   R  T  Q   Q  A  P>

      970        980        990       1000       1010       1020
    *   *    *   *    *   *    *   *    *   *    *   *    *   *
ATCCCTTGGT GAAACTAACG CCACAACAGG CCAACCAACT CAAGTCGAAT TCCGCCAGCG
TAGGGAACCA CTTTGATTGC GGTGTTGTCC GGTTGGTTGA GTTCAGCTTA AGGCGGTCGC
Y  P  L  V   K  L  T   P  Q  Q   A  N  Q  L   K  S  N   S  A  S>

      1030       1040       1050       1060       1070       1080

    *   *    *   *    *   *    *   *    *   *    *   *    *   *
TGAGCAGCCA GTACAACACT CTACCCAAAC TGGGCGACAC CAAGAGCCTG CACAGAGTGC
ACTCGTCGGT CATGTTGTGA GATGGGTTTG ACCCGCTGTG GTTCTCGGAC GTGTCTCACG
V  S  S  Q   Y  N  T   L  P  K   L  G  D  T   K  S  L   H  R  V>

      1090       1100       1110       1120       1130       1140
    *   *    *   *    *   *    *   *    *   *    *   *    *   *
CCATGACCAG GCAACAGCAC CCATTGCCCA CCAGCCATCA AGTGCAGCAG CAGCAGCATC
GGTACTGGTC CGTTGTCGTG GGTAACGGGT GGTCGGTAGT TCACGTCGTC GTCGTCGTAG
P  M  T  R   Q  Q  H   P  L  P   T  S  H  Q   V  Q  Q   Q  Q  H>

      1150       1160       1170       1180       1190       1200
    *   *    *   *    *   *    *   *    *   *    *   *    *   *
AGCTCCAGCA TCAACAGCAG CAGCAGCAGC AACATCATCA CCAGCATCAG CAACAACAGC
TCGAGGTCGT AGTTGTCGTC GTCGTCGTCG TTGTAGTAGT GGTCGTAGTC GTTGTTGTCG
Q  L  Q  H   Q  Q  Q   Q  Q  Q   Q  H  H  H   Q  H  Q   Q  Q  Q>

      1210       1220       1230       1240       1250       1260
    *   *    *   *    *   *    *   *    *   *    *   *    *   *
ATCAGCAACA GCAGCAACAT CAGATGCAGC ACCATCAGAT CCATCATCAG ACGGCGCCCA
TAGTCGTTGT CGTCGTTGTA GTCTACGTCG TGGTAGTCTA GGTAGTAGTC TGCCGCGGGT
H  Q  Q  Q   Q  Q  H   Q  M  Q   H  H  Q  I   H  H  Q   T  A  P>

      1270       1280       1290       1300       1310       1320
    *   *    *   *    *   *    *   *    *   *    *   *    *   *
GGAAGCCGCC CAAGAAGCAC AGCGAGATCT CCACCACCAA TCTACGCCAG ATACTCAACA
CCTTCGGCGG GTTCTTCGTG TCGCTCTAGA GGTGGTGGTT AGATGCGGTC TATGAGTTGT
R  K  P  P   K  K  H   S  E  I   S  T  T  N   L  R  Q   I  L  N>
```

FIG.12C

```
      1330       1340       1350       1360       1370       1380
    *    *     *    *     *    *     *    *     *    *     *    *
ACCTAAACAT CTTCAGCAGC AGCACTAAGC ACCAATCGAA CATGTCGACG GCGGCCAGTG
TGGATTTGTA GAAGTCGTCG TCGTGATTCG TGGTTAGCTT GTACAGCTGC CGCCGGTCAC
N  L  N  I  F  S  S   S  T  K  H  Q  S  N  M  S  T   A  A  S>

      1390       1400       1410       1420       1430       1440
    *    *     *    *     *    *     *    *     *    *     *    *
CCAGTTCATC CTCCTCATCG GCCTCGCTGC ACCATGCCAA CCATCTGTCG CATGCGCACT
GGTCAAGTAG GAGGAGTAGC CGGAGCGACG TGGTACGGTT GGTAGACAGC GTACGCGTGA
A  S  S  S   S  S  S   A  S  L   H  H  A  N   H  L  S   H  A  H>

      1450       1460       1470       1480       1490       1500
    *    *     *    *     *    *     *    *     *    *     *    *
TTTCGCACGC CAAGAACATG CTGACTGCCT CGATGAACAG TCATCATAGT CGCTGCTCGG
AAAGCGTGCG GTTCTTGTAC GACTGACGGA GCTACTTGTC AGTAGTATCA GCGACGAGCC
F  S  H  A   K  N  M   L  T  A   S  M  N  S   H  H  S   R  C  S>

      1510       1520       1530       1540       1550       1560
    *    *     *    *     *    *     *    *     *    *     *    *
AGGGATCGCT GCAGTCGCAA AGGAGCAGCC GGATGGGCTC GCATCGCTCG AGATCGCGAA
TCCCTAGCGA CGTCAGCGTT TCCTCGTCGG CCTACCCGAG CGTAGCGAGC TCTAGCGCTT
E  G  S  L   Q  S  Q   R  S  S   R  M  G  S   H  R  S   R  S  R>

      1570       1580       1590       1600       1610       1620
    *    *     *    *     *    *     *    *     *    *     *    *
CGCGGACCTC GGACACGGAC ACGAACAGTG TGAAATCGCA TCGGCGGAGA CCCAGTGTGG
GCGCCTGGAG CCTGTGCCTG TGCTTGTCAC ACTTTAGCGT AGCCGCCTCT GGGTCACACC
T  R  T  S   D  T  D   T  N  S   V  K  S  H   R  R  R   P  S  V>

      1630       1640       1650       1660       1670       1680
    *    *     *    *     *    *     *    *     *    *     *    *
ACACCGTGTC CACTTACCTC AGCCACGAGA GCAAGGAGAG CCTGCGCAGC AGGAACTTTG
TGTGGCACAG GTGAATGGAG TCGGTGCTCT CGTTCCTCTC GGACGCGTCG TCCTTGAAAC
D  T  V  S   T  Y  L   S  H  E   S  K  E  S   L  R  S   R  N  F>

      1690       1700       1710       1720       1730       1740
    *    *     *    *     *    *     *    *     *    *     *    *
CCATTTCGGT CAATGATCTG CTGGACTGCT CGCTTGGCAG CGATGAAGTT TTTGTGCCCT
GGTAAAGCCA GTTACTAGAC GACCTGACGA GCGAACCGTC GCTACTTCAA AAACACGGGA
A  I  S  V   N  D  L   L  D  C   S  L  G  S   D  E  V   F  V  P>
```

FIG.12D

```
      1750       1760       1770       1780       1790       1800
    *    *     *    *     *    *     *    *     *    *     *    *
CCGTGCCGCC ATCGTCGCTG GGCGAAAGGG CGCCGGTGCC GCCGCCATTA CCACTGCATC
GGCACGGCGG TAGCAGCGAC CCGCTTTCCC GCGGCCACGG CGGCGGTAAT GGTGACGTAG
S  V  P  P   S  S  L   G  E  R   A  P  V  P   P  P  L   P  L  H>

      1810       1820       1830       1840       1850       1860
    *    *     *    *     *    *     *    *     *    *     *    *
CGCGACAGCA ACAGCAGCAG CAACAACAGC AGCAACAGCT GCAGATGCAA CAGCAGCAAC
GCGCTGTCGT TGTCGTCGTC GTTGTTGTCG TCGTTGTCGA CGTCTACGTT GTCGTCGTTG
P  R  Q  Q   Q  Q  Q   Q  Q  Q   Q  Q  Q  L   Q  M  Q   Q  Q  Q>

      1870       1880       1890       1900       1910       1920
    *    *     *    *     *    *     *    *     *    *     *    *
AGGCGCAGCA GCAGCAGCAG CAATCAATCG CCGGTTCGAT TGTGGGCGTG GACCCGGCCA
TCCGCGTCGT CGTCGTCGTC GTTAGTTAGC GGCCAAGCTA ACACCCGCAC CTGGGCCGGT
Q  A  Q  Q   Q  Q  Q   Q  S  I   A  G  S  I   V  G  V   D  P  A>

      1930       1940       1950       1960       1970       1980
    *    *     *    *     *    *     *    *     *    *     *    *
GCGATATGAT ATCGCGTTTT GTCAAGGTGG TGGAGCCACC GCTGTGGCCC AATGCCCAGC
CGCTATACTA TAGCGCAAAA CAGTTCCACC ACCTCGGTGG CGACACCGGG TTACGGGTCG
S  D  M  I   S  R  F   V  K  V   V  E  P  P   L  W  P   N  A  Q>

      1990       2000       2010       2020       2030       2040
    *    *     *    *     *    *     *    *     *    *     *    *
CCTGTCCCAT GTGCATGGAG GAGCTGGTGC ACTCCGCCCA GAATCCGGCC ATTTCGCTGA
GGACAGGGTA CACGTACCTC CTCGACCACG TGAGGCGGGT CTTAGGCCGG TAAAGCGACT
P  C  P  M   C  M  E   E  L  V   H  S  A  Q   N  P  A   I  S  L>

      2050       2060       2070       2080       2090       2100
    *    *     *    *     *    *     *    *     *    *     *    *
GTCGCTGCCA GCATCTCATG CATTTGCAGT GCCTCAATGG GATGATAATT GCCCAGCAAA
CAGCGACGGT CGTAGAGTAC GTAAACGTCA CGGAGTTACC CTACTATTAA CGGGTCGTTT
S  R  C  Q   H  L  M   H  L  Q   C  L  N  G   M  I  I   A  Q  Q>

      2110       2120       2130       2140       2150       2160
    *    *     *    *     *    *     *    *     *    *     *    *
ACGAAATGAA CAAGAACCTT TTCATCGAGT GCCCTGTATG CGGCATCGTT TACGGCGAGA
TGCTTTACTT GTTCTTGGAA AAGTAGCTCA CGGGACATAC GCCGTAGCAA ATGCCGCTCT
N  E  M  N   K  N  L   F  I  E   C  P  V  C   G  I  V   Y  G  E>
```

FIG.12E

```
      2170       2180       2190       2200       2210       2220
    *    *     *    *     *    *     *    *     *    *     *    *
AGGTCGGCAA TCAGCCCATT GGCAGCATGT CGTGGAGCAT AATTAGCAAG AATCTGCCAG
TCCAGCCGTT AGTCGGGTAA CCGTCGTACA GCACCTCGTA TTAATCGTTC TTAGACGGTC
K  V  G  N   Q  P  I   G  S  M   S  W  S  I   I  S  K   N  L  P>

      2230       2240       2250       2260       2270       2280
    *    *     *    *     *    *     *    *     *    *     *    *
GACACGAGGG TCAGAACACC ATACAGATTG TTTACGACAT TGCATCGGGA CTGCAGACGG
CTGTGCTCCC AGTCTTGTGG TATGTCTAAC AAATGCTGTA ACGTAGCCCT GACGTCTGCC
G  H  E  G   Q  N  T   I  Q  I   V  Y  D  I   A  S  G   L  Q  T>

      2290       2300       2310       2320       2330       2340
    *    *     *    *     *    *     *    *     *    *     *    *
AGGAGCATCC GCATCCAGGT CGTGCCTTCT TCGCCGTGGG ATTCCCGCGG ATCTGCTACT
TCCTCGTAGG CGTAGGTCCA GCACGGAAGA AGCGGCACCC TAAGGGCGCC TAGACGATGA
E  E  H  P   H  P  G   R  A  F   F  A  V  G   F  P  R   I  C  Y>

      2350       2360       2370       2380       2390       2400
    *    *     *    *     *    *     *    *     *    *     *    *
TGCCGGACTG CCCGCTGGGG CGAAAGGTTT TGCGCTTCCT CAAGATTGCA TTCGATCGTC
ACGGCCTGAC GGGCGACCCC GCTTTCCAAA ACGCGAAGGA GTTCTAACGT AAGCTAGCAG
L  P  D  C   P  L  G   R  K  V   L  R  F  L   K  I  A   F  D  R>

      2410       2420       2430       2440       2450       2460
    *    *     *    *     *    *     *    *     *    *     *    *
GGCTGCTTTT CTCGATCGGA CGATCGGTGA CCACCGGACG CGAGGATGTG GTGATCTGGA
CCGACGAAAA GAGCTAGCCT GCTAGCCACT GGTGGCCTGC GCTCCTACAC CACTAGACCT
R  L  L  F   S  I  G   R  S  V   T  T  G  R   E  D  V   V  I  W>

      2470       2480       2490       2500       2510       2520
    *    *     *    *     *    *     *    *     *    *     *    *
ACAGTGTGGA TCACAAGACG CAGTTCAATA TGTTTCCGGA TCCCACCTAT TTGCAGCGAA
TGTCACACCT AGTGTTCTGC GTCAAGTTAT ACAAAGGCCT AGGGTGGATA AACGTCGCTT
N  S  V  D   H  K  T   Q  F  N   M  F  P  D   P  T  Y   L  Q  R>

      2530       2540       2550       2560       2570       2580
    *    *     *    *     *    *     *    *     *    *     *    *
CCATGCAACA GCTGGTGCAC CTGGGCGTGA CGGATTAAGG ATTAGTTCCC TGTCCCCAAC
GGTACGTTGT CGACCACGTG GACCCGCACT GCCTAATTCC TAATCAAGGG ACAGGGGTTC
T  M  Q  Q   L  V  H   L  G  V   T  D  *>
```

FIG.12F

```
      2590       2600       2610       2620       2630       2640
     *    *     *    *     *    *     *    *     *    *     *    *
TAGAACTACC AACCAACCAA TCAACCACCC ACCCACCGAA GTCCCCTCGA TCATTCTCTT
ATCTTGATGG TTGGTTGGTT AGTTGGTGGG TGGGTGGCTT CAGGGGAGCT AGTAAGAGAA

      2650       2660       2670       2680       2690       2700
     *    *     *    *     *    *     *    *     *    *     *    *
CCATTCGTCG TTAAGTTACT TTCTACATAA TCTCAGTGTG TGTGCAATCC TCGTTTACTA
GGTAAGCAGC AATTCAATGA AAGATGTATT AGAGTCACAC ACACGTTAGG AGCAAATGAT

      2710       2720       2730       2740       2750       2760
     *    *     *    *     *    *     *    *     *    *     *    *
TGATATATTT TTTTTATAGA TATATTGTAA TAGCGTTCGA GCTGCTCGAA CCCTAAAACA
ACTATATAAA AAAAATATCT ATATAACATT ATCGCAAGCT CGACGAGCTT GGGATTTTGT

      2770       2780       2790       2800       2810       2820
     *    *     *    *     *    *     *    *     *    *     *    *
ACAGCAAACC ACAATTGCAA TTGTAGCTTC CTTTCCGCTC TTCCAATTCG TATTTGTACG
TGTCGTTTGG TGTTAACGTT AACATCGAAG GAAAGGCGAG AAGGTTAAGC ATAAACATGC

      2830       2840       2850       2860       2870       2880
     *    *     *    *     *    *     *    *     *    *     *    *
CACATACGCA ATAAGTTGGC GTACATCATA TGTATTAGCT AGTTAGTTAG TTAGTTAGTT
GTGTATGCGT TATTCAACCG CATGTAGTAT ACATAATCGA TCAATCAATC AATCAATCAA

      2890       2900       2910       2920       2930       2940
     *    *     *    *     *    *     *    *     *    *     *    *
AGTTGTAGCT GTAGTTCCCA AGAGAATCTT GACCCAAGAC ACCTACTAGT ATTAGGCATT
TCAACATCGA CATCAAGGGT TCTCTTAGAA CTGGGTTCTG TGGATGATCA TAATCCGTAA

      2950       2960       2970       2980       2990       3000
     *    *     *    *     *    *     *    *     *    *     *    *
ATCCTGATTC TTGATTCCTG ATTCGATTCA AGCCAAGCCA AGCCACGCCA TTCGAGTGCA
TAGGACTAAG AACTAAGGAC TAAGCTAAGT TCGGTTCGGT TCGGTGCGGT AAGCTCACGT

      3010       3020       3030       3040       3050       3060
     *    *     *    *     *    *     *    *     *    *     *    *
AGCTGTGCCA AAATCGTAGC GCTCCCGTTT ATAGGATATG TATATTGTTG ATATAGCTAG
TCGACACGGT TTTAGCATCG CGAGGGCAAA TATCCTATAC ATATAACAAC TATATCGATC
```

FIG.12G

```
      3070       3080       3090       3100       3110       3120
    *    *     *    *     *    *     *    *     *    *     *    *
CTATAACCAT TGCCCATCTC TCCATCTCTC TCGGTTTCGA ATTTGTCTCT TTCATCAGAT
GATATTGGTA ACGGGTAGAG AGGTAGAGAG AGCCAAAGCT TAAACAGAGA AAGTAGTCTA

      3130       3140       3150       3160       3170       3180
    *    *     *    *     *    *     *    *     *    *     *    *
CCATGTGAAT TTTCTTTATA TCGGATTTAT ATAGGATTAA AATAGTATTT TGAGAGAGGA
GGTACACTTA AAAGAAATAT AGCCTAAATA TATCCTAATT TTATCATAAA ACTCTCTCCT

      3190       3200       3210       3220       3230       3240
    *    *     *    *     *    *     *    *     *    *     *    *
AATGGAGATG GGTAAATTCG ATAGACTTGT CTCACTTGTC TTGGCCATTT AATCTCTTTC
TTACCTCTAC CCATTTAAGC TATCTGAACA GAGTGAACAG AACCGGTAAA TTAGAGAAAG

      3250       3260       3270       3280       3290       3300
    *    *     *    *     *    *     *    *     *    *     *    *
ATTCAGCGAA TTTGATGTGA TTTTAATTTG AATTATTCAT TATTAAACGG AGCATTTAGG
TAAGTCGCTT AAACTACACT AAAATTAAAC TTAATAAGTA ATAATTTGCC TCGTAAATCC

      3310       3320       3330       3340       3350       3360
    *    *     *    *     *    *     *    *     *    *     *    *
AAGCATAGTT GTAACGCAGC CAGATATTCC ATTACGCATA TACATATACA TATACATATA
TTCGTATCAA CATTGCGTCG GTCTATAAGG TAATGCGTAT ATGTATATGT ATATGTATAT

      3370       3380       3390       3400       3410       3420
    *    *     *    *     *    *     *    *     *    *     *    *
CATACATACA TAAACATATT TTAACATAGC CCCATAGCCA TACGACATAA CAATAATTTT
GTATGTATGT ATTTGTATAA AATTGTATCG GGGTATCGGT ATGCTGTATT GTTATTAAAA

      3430       3440       3450       3460       3470       3480
    *    *     *    *     *    *     *    *     *    *     *    *
TTTTATCGAA TCCCTTGCAT ACATTTGATG AATTGTTGCT TTCATATTGA TATCATCGAG
AAAATAGCTT AGGGAACGTA TGTAAACTAC TTAACAACGA AAGTATAACT ATAGTAGCTC

      3490       3500       3510       3520       3530       3540
    *    *     *    *     *    *     *    *     *    *     *    *
CATCGAACGA ACTATCGTAT ACATCGCCAA TATATAGCAT ATATAGCATA TAGTATGTAG
GTAGCTTGCT TGATAGCATA TGTAGCGGTT ATATATCGTA TATATCGTAT ATCATACATC
```

FIG.12H

```
      3550        3560        3570        3580        3590        3600
     *   *       *   *       *   *       *   *       *   *       *   *
AGATCGTACG GACAGCTAGC GGCTACTGAC CGCGCCACCA TATTTGATAT GATATGATAT
TCTAGCATGC CTGTCGATCG CCGATGACTG GCGCGGTGGT ATAAACTATA CTATACTATA

      3610        3620        3630        3640        3650        3660
     *   *       *   *       *   *       *   *       *   *       *   *
GATTTTACTA AGTTGTATTT AGCACTGATT AGTTATTAAA GTTCATTTGA CGAATATTCC
CTAAAATGAT TCAACATAAA TCGTGACTAA TCAATAATTT CAAGTAAACT GCTTATAAGG

      3670        3680        3690        3700        3710        3720
     *   *       *   *       *   *       *   *       *   *       *   *
ACAACAAATT CCACACCATT TATGTATGCA TATTACGCAT ATATAATACA GTACATTTAT
TGTTGTTTAA GGTGTGGTAA ATACATACGT ATAATGCGTA TATATTATGT CATGTAAATA

      3730        3740        3750        3760        3770
     *   *       *   *       *   *       *   *       *   *
ATATAGTTCA AATAAAGTAA CTTCATTCAT GTTCAAAAAA AAAAAAAAAA A
TATATCAAGT TTATTTCATT GAAGTAAGTA CAAGTTTTTT TTTTTTTTTT T
```

FIG.12I

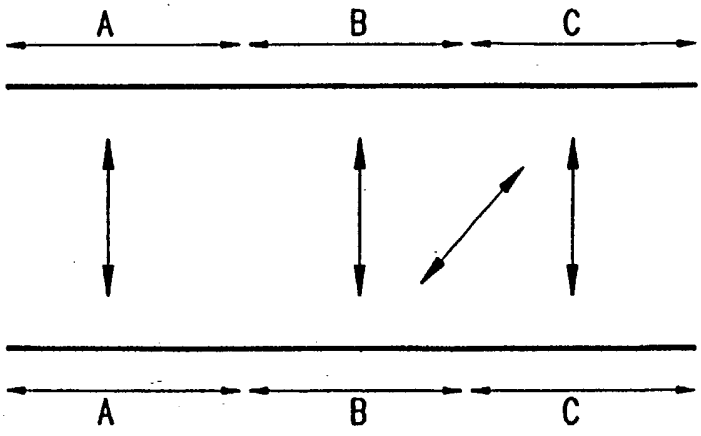
A
B
C
A
B
C

FIG.13

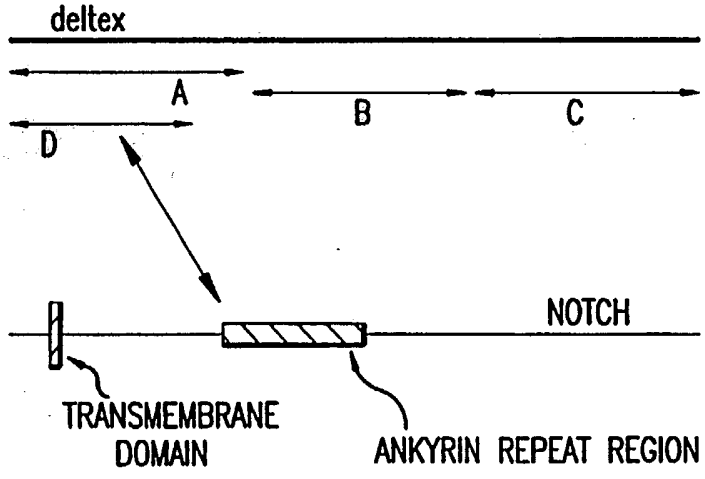
deltex
A
B
C
D
NOTCH
TRANSMEMBRANE DOMAIN
ANKYRIN REPEAT REGION

FIG.15

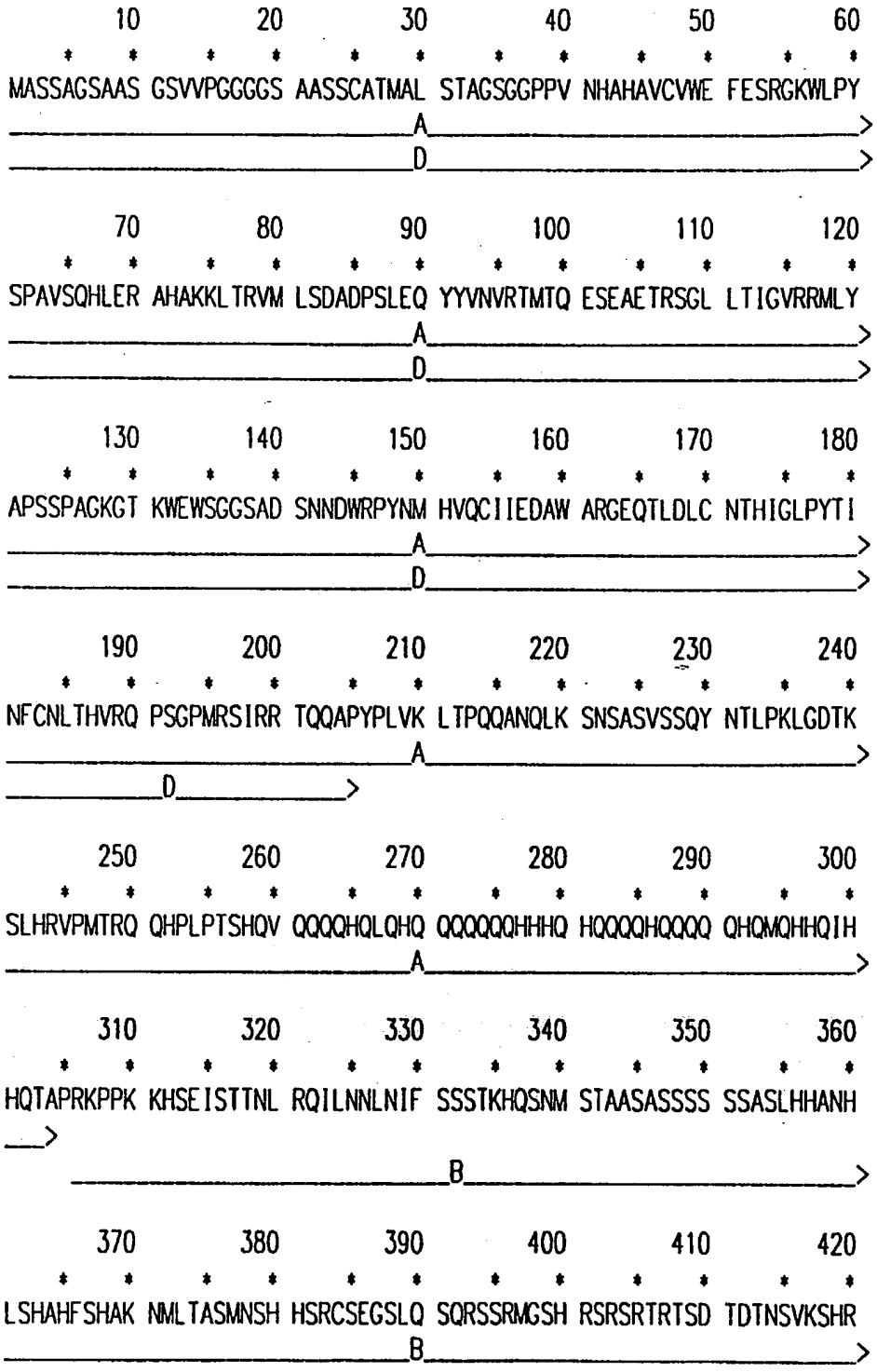
10 20 30 40 50 60
MASSAGSAAS GSVVPGGGGS AASSCATMAL STAGSGGPPV NHAHAVCVWE FESRGKWLPY
A
D
70 80 90 100 110 120
SPAVSQHLER AHAKKLTRVM LSDADPSLEQ YYVNVRTMTQ ESEAETRSGL LTIGVRRMLY
A
D
130 140 150 160 170 180
APSSPAGKGT KWEWSGGSAD SNNDWRPYNM HVQCIIEDAW ARGEQTLDLC NTHIGLPYTI
A
D
190 200 210 220 230 240
NFCNLTHVRQ PSGPMRSIRR TQQAPYPLVK LTPQQANQLK SNSASVSSQY NTLPKLGDTK
A
D
250 260 270 280 290 300
SLHRVPMTRQ QHPLPTSHQV QQQQHQLQHQ QQQQQQHHHQ HQQQQHQQQQ QHQMQHHQIH
A
310 320 330 340 350 360
HQTAPRKPPK KHSEISTTNL RQILNNLNIF SSSTKHQSNM STAASASSSS SSASLHHANH
B
370 380 390 400 410 420
LSHAHFSHAK NMLTASMNSH HSRCSEGSLQ SQRSSRMGSH RSRSRTRTSD TDTNSVKSHR
B

FIG.14A

```
      430       440       450       460       470       480
    *   *     *   *     *   *     *   *     *   *     *   *
RRPSVDTVST YLSHESKESL RSRNFAISVN DLLDCSLGSD EVFVPSVPPS SLGERAPVPP
_____________________________B_____________________________>

      490       500       510       520       530       540
    *   *     *   *     *   *     *   *     *   *     *   *
PLPLHPRQQQ QQQQQQQQLQ MQQQQQAQQQ QQQSIAGSIV GVDPASDMIS RFVKVVEPPL
_____>
                                          _____C_____>

      550       560       570       580       590       600
    *   *     *   *     *   *     *   *     *   *     *   *
WPNAQPCPMC MEELVHSAQN PAISLSRCQH LMHLQCLNGM IIAQQNEMNK NLFIECPVCG
_____________________________C_____________________________>

      610       620       630       640       650       660
    *   *     *   *     *   *     *   *     *   *     *   *
IVYGEKVGNQ PIGSMSWSII SKNLPGHEGQ NTIQIVYDIA SGLQTEEHPH PGRAFFAVGF
_____________________________C_____________________________>

      670       680       690       700       710       720
    *   *     *   *     *   *     *   *     *   *     *   *
PRICYLPDCP LGRKVLRFLK IAFDRRLLFS IGRSVTTGRE DVVIWNSVDH KTQFNMFPDP
_____________________________C_____________________________>

      730
    *   *     *
TYLQRTMQQL VHLGVTD
_____C_____>
```

FIG.14B

	REGION	CORE	SEQ. ID NO.
MOUSE 3BP-1 (GAP)		RAPTMPPPLPPVPPQP	9
FLY *DELTEX*	1 (58aa) 2	RAP-VPPPLPLHPRQQ... FVKVVEPPLWPNA-QP	10 11
FLY *SON OF SEVENLESS* (GNEF)	1 (37aa) 2	RA—VPPPLPPRRKER DAPTLPPR DGELSPPPIPPRLNHS	12 13
FLY *HAIRLESS*	1	SYPPLPPPLPANLSRT	14
FLY *DISABLED*	1	SVDAPPIPLPSRRVGR	15

FIG.16

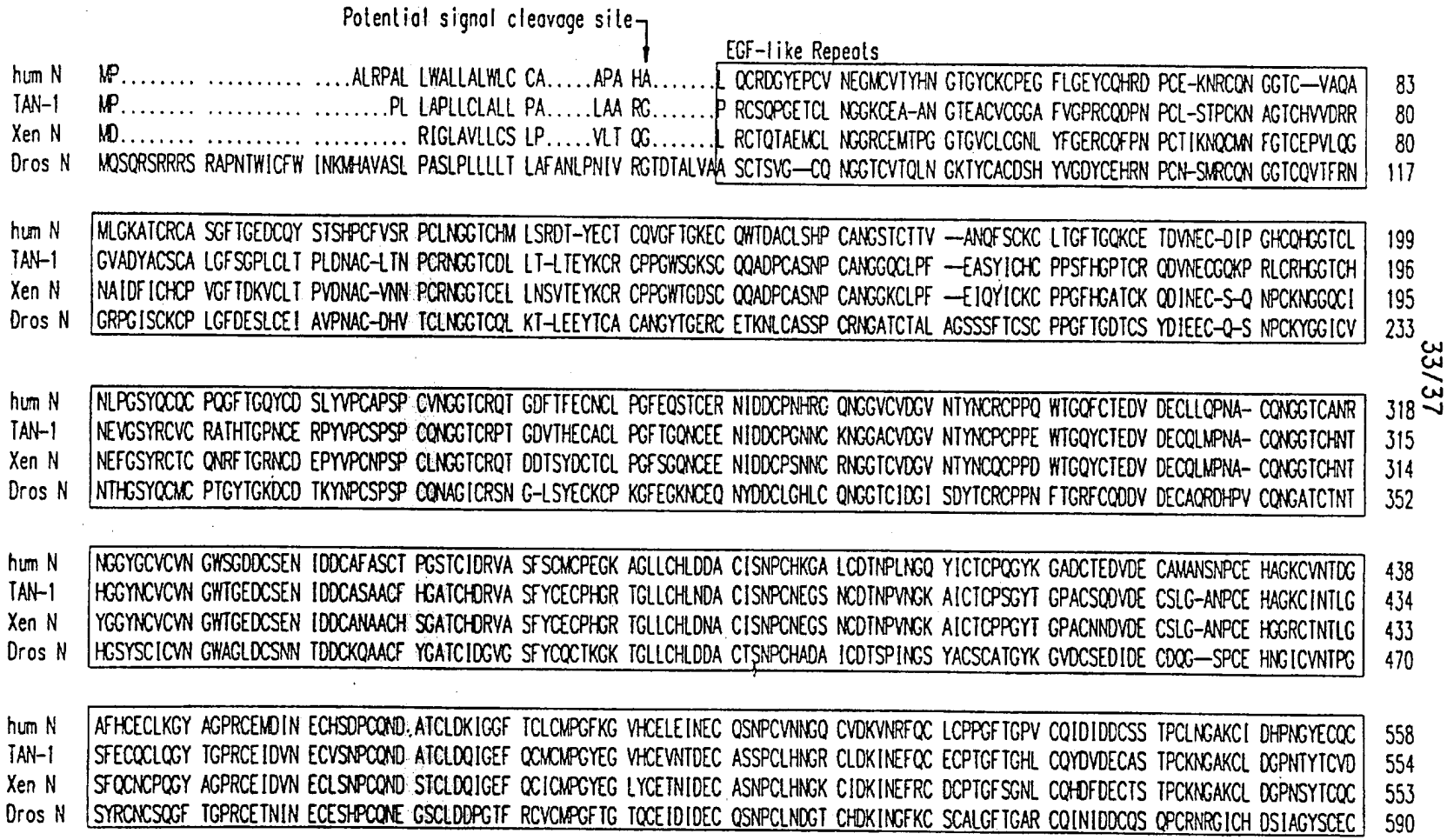
Potential signal cleavage site
EGF-like Repeats
hum N MP..... ....ALRPAL LWALLALWLC CA.....APA HA.....L QCRDGYEPCV NEGMCVTYHN GTGYCKCPEG FLGEYCQHRD PCE-KNRCQN GGTC—VAQA 83
TAN-1 MP..... ....PL LAPLLCLALL PA.....LAA RG.....P RCSQPGETCL NGGKCEA-AN GTEACVCGGA FVGPRCQDPN PCL-STPCKN AGTCHVVDRR 80
Xen N MD..... .... RIGLAVLLCS LP.....VLT QG.....L RCTQTAEMCL NGGRCEMTPG GTGVCLCGNL YFGERCQFPN PCTIKNQCMN FGTCEPVLQG 80
Dros N MQSQRSRRRS RAPNTWICFW INKMHAVASL PASLPLLLLT LAFANLPNIV RGTDTALVAA SCTSVG—CQ NGGTCVTQLN GKTYCACDSH YVGDYCEHRN PCN-SMRCQN GGTCQVTFRN 117
hum N MLGKATCRCA SGFTGEDCQY STSHPCFVSR PCLNGGTCHM LSRDT-YECT CQVGFTGKEC QWTDACLSHP CANGSTCTTV —ANQFSCKC LTGFTGQKCE TDVNEC-DIP GHCQHGGTCL 199
TAN-1 GVADYACSCA LGFSGPLCLT PLDNAC-LTN PCRNGGTCDL LT-LTEYKCR CPPGWSGKSC QQADPCASNP CANGGQCLPF —EASYICHC PPSFHGPTCR QDVNECGQKP RLCRHGGTCH 196
Xen N NAIDFICHCP VGFTDKVCLT PVDNAC-VNN PCRNGGTCEL LNSVTEYKCR CPPGWTGDSC QQADPCASNP CANGGKCLPF —EIQYICKC PPGFHGATCK QDINEC-S-Q NPCKNGGQCI 195
Dros N GRPGISCKCP LGFDESLCEI AVPNAC-DHV TCLNGGTCQL KT-LEEYTCA CANGYTGERC ETKNLCASSP CRNGATCTAL AGSSSFTCSC PPGFTGDTCS YDIEEC-Q-S NPCKYGGICV 233
hum N NLPGSYQCQC PQGFTGQYCD SLYVPCAPSP CVNGGTCRQT GDFTFECNCL PGFEQSTCER NIDDCPNHRC QNGGVCVDGV NTYNCRCPPQ WTGQFCTEDV DECLLQPNA- CQNGGTCANR 318
TAN-1 NEVGSYRCVC RATHTGPNCE RPYVPCSPSP CQNGGTCRPT GDVTHECACL PGFTGQNCEE NIDDCPGNNC KNGGACVDGV NTYNCPCPPE WTGQYCTEDV DECQLMPNA- CQNGGTCHNT 315
Xen N NEFGSYRCTC QNRFTGRNCD EPYVPCNPSP CLNGGTCRQT DDTSYDCTCL PGFSGQNCEE NIDDCPSNNC RNGGTCVDGV NTYNCQCPPD WTGQYCTEDV DECQLMPNA- CQNGGTCHNT 314
Dros N NTHGSYQCMC PTGYTGKDCD TKYNPCSPSP CQNAGICRSN G-LSYECKCP KGFEGKNCEQ NYDDCLGHLC QNGGTCIDGI SDYTCRCPPN FTGRFCQDDV DECAQRDHPV CQNGATCTNT 352
hum N NGGYGCVCVN GWSGDDCSEN IDDCAFASCT PGSTCIDRVA SFSCMCPEGK AGLLCHLDDA CISNPCHKGA LCDTNPLNGQ YICTCPQGYK GADCTEDVDE CAMANSNPCE HAGKCVNTDG 438
TAN-1 HGGYNCVCVN GWTGEDCSEN IDDCASAACF HGATCHDRVA SFYCECPHGR TGLLCHLNDA CISNPCNEGS NCDTNPVNGK AICTCPSGYT GPACSQDVDE CSLG-ANPCE HAGKCINTLG 434
Xen N YGGYNCVCVN GWTGEDCSEN IDDCANAACH SGATCHDRVA SFYCECPHGR TGLLCHLDNA CISNPCNEGS NCDTNPVNGK AICTCPPGYT GPACNNDVDE CSLG-ANPCE HGGRCTNTLG 433
Dros N HGSYSCICVN GWAGLDCSNN TDDCKQAACF YGATCIDGVG SFYCQCTKGK TGLLCHLDDA CTSNPCHADA ICDTSPINGS YACSCATGYK GVDCSEDIDE CDQG—SPCE HNGICVNTPG 470
hum N AFHCECLKGY AGPRCEMDIN ECHSDPCQND.ATCLDKIGGF TCLCMPGFKG VHCELEINEC QSNPCVNNGQ CVDKVNRFQC LCPPGFTGPV CQIDIDDCSS TPCLNGAKCI DHPNGYECQC 558
TAN-1 SFECQCLQGY TGPRCEIDVN ECVSNPCQND ATCLDQIGEF QCMCMPGYEG VHCEVNTDEC ASSPCLHNGR CLDKINEFQC ECPTGFTGHL CQYDVDECAS TPCKNGAKCL DGPNTYTCVD 554
Xen N SFQCNCPQGY AGPRCEIDVN ECLSNPCQND STCLDQIGEF QCICMPGYEG LYCETNIDEC ASNPCLHNGK CIDKINEFRC DCPTGFSGNL CQHDFDECTS TPCKNGAKCL DGPNSYTCQC 553
Dros N SYRCNCSQGF TGPRCETNIN ECESHPCQNE GSCLDDPGTF RCVCMPGFTG TQCEIDIDEC QSNPCLNDGT CHDKINGFKC SCALGFTGAR CQINIDDCQS QPCRNRGICH DSIAGYSCEC 590

FIG.17A

```
hum N   ATGFTGVLCE ENIDNCDPDP CHHGQCQDGI DSYTCICNPG YMGAICSDQI DECYSSPCLN DGRCIDLVNG YQCNCQPGTS GVNCEINFDD CASNPCIHG- ICMDGINRYS CVCSPGFTGQ  677
TAN-1   TEGYTGTHCE VDIDECDPDP CHYGSCKDGV ATFTCLCRPG YTGHHCETNI NECSSQPCRL RGTCQDPDNA YLCFCLKGTT GPNCEINLDD CASSPCDSG- TCLDKIDGYE CACEPGYTGS  673
Xen N   TEGFTGRHCE QDINECIPDP CHYGTCKDGI ATFTCLCRPG YTGRLCDNDI NECLSKPCLN GGQCTDRENG YICTCPKGTT GVNCETKIDD CASNLCDNG- KCIDKIDGYE CTCEPGYTGK  672
Dros N  PPGYTGTSCE ININDCDSNP CHRGKCIDDV NSFKCLCDPG YTGYICQKQI NECESNPCQF DGHCQDRVGS YYCQCQAGTS GKNCEVNVNE CHSNPCNNGA TCIDGINSYK CQCVPGFTGQ  710

hum N   RCNIDIDECA SNPCRKGATC INGVNGFRCI CPEGPHHPSC YSQVNECLSN PCI-HGNCTG GLSGYKCLCD AGWVGINCEV DKNECLSNPC QNGGTCDNLV NGYRCTCKKG FKGYHCQVNI  796
TAN-1   MCNSNIDECA GNPCHNGGTC EDGINGFTCR CPEGYHDPTC LSEVNECNSN PCV-HGACRD SLNGYKCDCD PGWSGTNCDI NNNECESNPC VNGGTCKDMT SGIVCTCREG FSGPHCQTNI  792
Xen N   LCNININECD SNPCRNGGTC KDQINGFTCV CPDGYHDHMC LSEVNECNSN PCI-HGACHD GVNGYKCDCE AGWSGSNCDI NNNECESNPC MNGGTCKDMT GAYICTCKAG FSGPNCQTNI  791
Dros N  HCEKNVDECI SSPCANNGVC IDQVNGYKCE CPRGFYDAHC LSDVDECASN PCVNEGRCED GINEFICHCP PGYTGKRCEL DIDECSSNPC QHGGTCYDKL NAFSCQCMPG YTGQKCETNI  830

hum N   DECASNPCLN QGTCFDDISG YTCHCVLPYT GKNCQTVLAP CSPNPCENAA VCKESPNFES YTCLCA-PGW QGQRCTIDID EC-ISKPCMN HGLCHNTQGS YMCECPPGFS GMDCEEDIDD  914
TAN-1   NECASNPCLN KGTCIDDVAG YKCNCLLPYT GATCEVVLAP CAPSPCRNGG ECRQSEDYES FSCVCPTAGA KGQTCEVDIN EC-VLSPCRH GASCQNTHGG YRCHCQAGYS GRNCETDIDD  911
Xen N   NECSSNPCLN HGTCIDDVAG YKCNCMLPYT GAICEAVLAP CAGSPCKNGG RCKESEDFET FSCECP-PGW QGQTCEIDMN EC-VNRPCRN GATCQNTNGS YKCNCKPGYT GRNCEMDIDD  909
Dros N  DDCVTNPCGN GGTCIDKVNG YKCVCKVPFT GRDCESKMDP CASNRCKNEA KCTPSSNFLD FSCTCK-LGY TGRYCDEDID ECSLSSPCRN GASCLNVPGS YRCLCTKGYE GRDCAINTDD  949

hum N   CLANPCQNGG SCMDGVNTFS CLCLPGFTGD KCQTDMNECL SEPCKNGGTC SDYVNSYTCK CQAGFDGVHC ENNINECTES SCFNGGTCVD GINSFSCLCP VGFTGSFCLH EINECSSHPC  1034
TAN-1   CRPNPCHNGG SCTDGINTAF CDCLPGFRGT FCEEDINECA SDPCRNGANC TDCVDSYTCT CPAGFSGIHC ENNTPDCTES SCFNGGTCVD GINSFTCLCP PGFTGSYCQH VVNECDSRPC  1031
Xen N   CQPNPCHNGG SCSDGINMFF CNCPAGFRGP KCEEDINECA SNPCKNGANC TDCVNSYTCT CQPGFSGIHC ESNTPDCTES SCFNGGTCID GINTFTCQCP PGFTGSYCQH DINECDSKPC  1029
Dros N  CASFPCQNGG TCLDGIGDYS CLCVDGFDGK HCETDINECL SQPCQNGATC SQYVNSYTCT CPLGFSGINC QTNDEDCTES SCLNGGSCID GINGYNCSCL AGYSGANCQY KLNKCDSNPC  1069

hum N   LNEGTCVDGL GTYRCSCPLG YTGKNCQTLV NLCSRSPCKN KGTCVQKKAE SQCLCPSGWA GAYCDVPNVS CDIAASRRGV LVEHLCQHSG VCINAGNTHY CQCPLGYTGS YCEEQLDECA  1154
TAN-1   LLGGTCQDGR GLHRCTCPQG YTGPNCQNLV HWCDSSPCKN GGKCWQTHTQ YRCECPSGWT GLYCDVPSVS CEVAAQRQGV DVARLCQHGG LCVDAGNTHH CRCQAGYTGS YCEDLVDECS  1151
Xen N   LNGGTCQDSY GTYKCTCPQG YTGLNCQNLV RWCDSSPCKN GGKCWQTNNF YRCECKSGWT GVYCDVPSVS CEVAAKQQGV DIVHLCRNSG MCVDTGNTHF CRCQAGYTGS YCEEQVDECS  1149
Dros N  LNGATCHEQN NEYTCHCPSG FTGKQCSEYV DWCGQSPCEN GATCSQMKHQ FSCKCSAGWT GKLCDVQTIS CQDAADRKGL SLRQLC-NNG TCKDYGNSHV CYCSQGYAGS YCQKEIDECQ  1188
```

FIG.17B

```
hum N   SNPCQHGATC SDFIGGYRCE CVPGYQGVNC EYEVDECQNQ PCQNGGTCID LVNHFKCSCP PGTRGLLCEE NIDDCAR--- ---GPHCLN GGQCMDRIGG YSCRCLPGFA GERCEGDINE 1267
TAN-1   PSPCQNGATC TDYLGGYSCK CVAGYHGVNC SEEIDECLSH PCQNGGTCLD LPNTYKCSCP RGTQGVHCEI NVDDCNPPVD PVSRSPKCFN NGTCVDQVGG YSCTCPPGFV GERCEGDVNE 1271
Xen N   PNPCQNGATC TDYLGGYSCE CVAGYHGVNC SEEINECLSH PCQNGGTCID LINTYKCSCP RGTQGVHCEI NVDDCTPFYD SFTLEPKCFN NGKCIDRVGG YNCICPPGFV GERCEGDVNE 1269
Dros N  SQPCQNGGTC RDLIGAYECQ CRQGFQGQNC ELNIDDCAPN PCQNGGTCHD RVMNFSCSCP PGTHGIICEI NKDDCKP--- ---GACHN NGSCIDRVGG FECVCQPGFV GARCEGDINE 1300

hum N   CLSNPCSSEG SLDCIQLTND YLCVCRSAFT GRHCETFVDV CPQMPCLNGG TCAVASNMPD GFICRCPPGF SGARCQS--- SCGQVKCRKG EQCVHTAS-- GPRCFCPSP- --RDCES--- 1376
TAN-1   CLSNPCDARG TQNCVQRVND FHCECRAGHT GRRCESVING CKGKPCKNGG TCAVASNTAR GFICKCPAGF EGATCENDAR TCGSLRCLNG GTCISGPR-- SPTCLCLGPF TGPECQFPAS 1389
Xen N   CLSNPCDSRG TQNCIQLVND YRCECRQGFT GRRCESVVDG CKGMPCRNGG TCAVASNTER GFICKCPPGF DGATCEYDSR TCSNLRCQNG GTCISVLT-- SSKCVCSEGY TGATCQYPVI 1387
Dros N  CLSNPCSNAG TLDCVQLVNN YHCNCRPGHM GRHCEHKVDF CAQSPCQNGG NCNI---RQS GHHCICNNGF YGKNCELSGQ DCDSNPCRVG -NCVVADEGF GYRCECPRGT LGEHCEIDTL 1415

                                                                                                LNR(Notch/Lin-12 Repeats
hum N   -GC-ASSPCQ HGGSCHPQRQ PPYYSCQCAP PFSGSRCEL- -YTAPP---- -S-----TPP ---A---TCL SQYCADKARD GVCDEACNSH ACQWDGGDCS LTMENPWANC SSPLPCWDYI 1476
TAN-1   SPCLGGNPCY NQGTCEPTSE SPFYRCLCPA KFNGLLCHIL DYSFGG---- -GAGRDIPPP LIEE---ACE LPECQEDAGN KVCSLQCNNH ACGWDGGDCS LNFNDPWKNC TQSLQCWKYF 1501
Xen N   SPC-ASHPCY NGGTCQFFAE EPFFQCFCPK NFNGLFCHIL DYEFPG---- -GLGKNITPP DNDD---ICE NEQCSELADN KVCNANCNNH ACGWDGGDCS LNFNDPWKNC TQSLQCWKYF 1498
Dros N  DEC-SPNPCA QGAACEDLLG D--YECLCPS KWKGKRCDIY DANYPGWNGG SGSGNDRYAA DLEQQRAMCD KRGCTEKQGN GICDSDCNTY ACNFDGNDCS LGI-NPWANC TAN-ECWNKF 1531

hum N   NN-QCDELCN TVECLFDNFE CQGNSKTCK- -YDKYCADHF KDNHCNQGCN SEECGWDGLD CAADQPEN-L AEGTLVIVVL MPPEQLLQDA R-SFLRALGT LLHTNLRIKR DSQGELMVYP 1591
TAN-1   SDGHCDSQCN SAGCLFDGFD CQRAEGQCNP LYDQYCKDHF SDGHCDQGCN SAECEWDGLD CAEHVPER-L AAGTL-VVVV LMPPEQLRNS SFHFLRELSR VLHTNVVFKR DAHGQQMIFP 1619
Xen N   NDGKCDSQCN NTGCLYDGFD CQKVEVQCNP LYDQYCKDHF QDGHCDQGCN NAECEWDGLD C-ANMPEN-L AEGTLVLVVL MPPERLKNNS V-NFLRELSR VLHTNVVFKK DSKGEYKIYP 1615
Dros N  KNGKCNEECN NAACHYDGHD CERKLKSCDS LFDAYCQKHY GDGFCDYGCN NAECSWDGLD CENKTQSPVL AEGAMSVVML MNVEAFREIQ A-QFLRNMSH MLRTTVRLKK DALGHDIIIN 1650

                                                                                                                              TM
hum N   YYGEKSAAMK KQ--R----- ---------- ---MTRRSL PGEQ-----E QEVAGSKVFL EIDNRQCVQD SDHCFKNTDA AAALLASHAI QG---TLSYP LVSVVSESLT PERT-Q-LLY 1680
TAN-1   YYGREEELRK HPIKRAAEGW AAPDALLGQV KASLLPGGSE GGRRRRELDP MDVRGSIVYL EIDNRQCVQA SSQCFQSATD VAAFLGALAS LGSL-NIPYK IEAVQSETVE PPPPAQ-LHF 1737
Xen N   YYGNEEELKK HHIKRSTDYW SDAPSAI--- -FSTMKESIL LGRHRRELDE MEVRGSIVYL EIDNRQCYKS SSQCFNSATD VAAFLGALAS LGSLDTLSYK IEAVKSENME TPKPST-LYP 1730
Dros N  WKDNVRVPEI EDTDFARKNK ILYTQQVHQ- ---------- ---------- --TGIQIYL EIDNRKCTEC FTHAVEAAEF LAATAAKHQL RNDFQ-IHSV RGIKNPGDED NGEPPANVKY 1745
```

FIG.17C

```
hum N   LLAVAVVIIL FIILLGVIMA KRKRK--HGS LWLPEGFTLR RDASNHKRRE PVGQDAVGLK NLSVQVSEAN LIGTGTSEHW VDDE------ ---------G PQPKKVKAED EALLSE-EDD 1782
TAN-1   MYVAAAAFVL LFFVGCGVLL SRKRRRQHGQ LWFPEGFKV- SEASKKKRRE ELGEDSVGLK PLK-NASDGA LMDDNQNE-W GDED------ ---------- LETKKFRFEE PVVLPD-LDD 1837
Xen N   MLSMLVIPLL IIFVFMMVIV NKKRRREHDS FGSPTALFQK NPA-KRNGET PW-EDSVGLK PIK-NMTDGS FMDDNQNE-W GDEET----- ---------- LENKRFRFEE QVILPELVDD 1831
Dros N  VITGIILVII ALAFFGMVL- STQRKRAHGV TWFPEGFRAP AAVMSRRRRD PHGQEMRNLN KQVAMQSQGV GQPGAH---W SDDESDMPLP KRQRSDPVSG VGLGNNGGYA SDHTMVSEYE 1861

                                    CDC-10/Ankyrin Repeats
hum N   PIDRRPWTQQ HLEAADIRRT PSLALTPPQA EQEVDVLDVN VRGPDGCTPL MLASLRGGSS DLSDEDEDAE DSSANIITDL VYQGASLQAQ TDRTGEMALH LAARYSRADA AKRLLDAGAD 1902
TAN-1   QTDHRQWTQQ HLDAADL-RM SAMAPTPPQG EVDADCMDVN VRGPDGFTPL MIASCSGGGL ETGNSEEE-E DAPA-VISDF IYQGASLHNQ TDRTGETALH LAARYSRSDA AKRLLEASAD 1954
Xen N   KTDPRQWTRQ HLDAADL-RI SSMAPTPPQG EIEADCMDVN VRGPDGFTPL MIASCSGGGL ETGNSEEE-E DASANMISDF IGQGAQLHNQ TDRTGETALH LAARYARADA AKRLLESSAD 1949
Dros N  EADQRVWSQA HLDVVDV-R- AIM--TPP-A HQDGGKHDVD ARGPCGLTPL MIAAVRGGGL DTGEDIENNE DSTAQVISDL LAQGAELNAT MDKTGETSLH LAARFARADA AKRLLDAGAD 1976

hum N   ANAQDNMGRC PLHAAVAADA QGVFQILIRN RVTDLDARMN DGTTPLILAA RLAVEGMVAE LINCQADVNA VDDHGKSALH WAAAVNNVEA TLLLLKNGAN RDMQDNKEET PLFLAAREGS 2022
TAN-1   ANIQDNMGRT PLHAAVSADA QGVFQILIRN RATDLDARMH DGTTPLILAA RLAVEGMLED LINSHADVNA VDDLGKSALH WAAAVNNVDA AVVLLKNGAN KDMQNNREET PLFLAAREGS 2074
Xen N   ANVQDNMGRT PLHAAVAADA QGVFQILIRN RATDLDARMF DGTTPLILAA RLAVEGMVEE LINAHADVNA VDEFGKSALH WAAAVNNVDA AAVLLKNSAN KDMQNNKEET SLFLAAREGS 2069
Dros N  ANCQDNTGRT PLHAAVAADA MGVFQILLRN RATNLNARMH DGTTPLILAA RLAIEGMVED LITADADINA ADNSGKTALH WAAAVNNTEA VNILLMHHAN RDAQDDKDET PLFLAAREGS 2096

hum N   YEAAKILLDH FANRDITDHM DRLPRDVARD RMHHDIVRLL DEYNVTPSPP -GTVL--TS ALSPV----- ------ICGP NRSFLSLKHT PHGKKSRRPS AKSTMPTSLP NLAKEAKDAK 2127
TAN-1   YETAKVLLDH FANRDITDHM DRLPRDIAQE RMHHDIVRLL DEYNLVRSPQ LHGAPLGGTP TLSPP----- ------LCSP NGYLGSLKPG VQGKKVRKPS SKGLACGS-- --KEAKDLK 2178
Xen N   YETAKVLLDH YANRDITDHM DRLPRDIAQE RMHHDIVHLL DEYNLVKSPT LHNGPLGAT- TLSPP----- ------ICSP NGYMGNMKPS VQSKKARKPS IKGNGC---- --KEAKELK 2170
Dros N  YEACKALLDN FANREITDHM DRLPRDVASE RLHHDIVRLL DE-HVPRSPQ MLSMTPQAMI GSPPPGQQQP QLITQPTVIS AGNGGNNGNG NASGKQSNQT AKQKAA---- --KKAKLIE 2208

           NLS                   CK II     cdc2       cdc2
hum N   GSRRKKGLSE KVQLSE--SS VTLSPVDSLE SPHTYVSDTT SSPM------ ---------- ---------- ---------- ---------- ---------- ---------- 2169
TAN-1   A-RRKKSQDG KGCLLD--SS GMLSPVDSLE SPHGYLSDVA SPPL------ ---------- ---------- ---------- ---------- ---------- ---------- 2219
Xen N   A-RRKKSQDG KTTLLDSGSS GVLSPVDGLE STHGYLSDVS SPPL------ ---------- ---------- ---------- ---------- ---------- ---------- 2213
Dros N  GS-PDNGLDA TGSLRRKASS KKTSAASKKA ANLNGLNPGQ LTGGVSGVPG VPPTNSAAQA AAAAAAAVAA MSHELEGSPV GVGMGGNLPS PYDTSSMYSN AMAAPLANGN PNTGAKQPPS 2327
        BNTS
```

FIG.17D

```
hum N  ———— ———— ———— ITSPGILQAS PNPML—ATA APPAPVHAQH ALSFSNLHEM Q———— ———— -PLAHGASTV LPSVSQLLSH HHIVSPGS—  2235
TAN-1  ———— ———— ———— LFSPF—QQS PSVPLNHLPG MPDTHLGIGH LNVAA-KPEM AALGGGGRLA FETGPPRLSH LPVASGTSTV LGSSSGGALN FTVGGSTSLN 2306
Xen N  ———— ———— ———— MTSPF—QQS PSMPLNHLTS MPESQLGMNH INMAT-KQEM AA—GSNRMA FDAMVPRLTH L-NASSPNTI MS——NGSMH FTVGGAPTMN 2294
Dros N YECCIKNAQS MQSLQGNGLD MIKLDNYAYS MQSPF—QQE LLNGQGLGMN GNGQRNGVGP GVLPGGLCGH GGLSGAGNGN SHEQGLSPPY SNQSPPHSVQ SSLALSPHAY LGSPSPAKSR 2445
       CK II                  cdc2

hum N  GSAGSLSRLH PVPVPADW— MNRMEVNETQ YNEMFGMVLA PAEG-THPGI APQSRPPEGK ———— ——HITTPRE PLPP-IV-TF QLIPKGSIAQ PAG———— ———— 2320
TAN-1  GQCEWLSRLQ SGMVPNQYNP LRGSVAPGPL STQAPSLQHG -MVGPLHSSL AASALSQMMS ———— -YQGLPSTRL ATQPHLVQTQ QVQPQNLQMQ QQNLQPANIQ QQQSLQPPPP 2414
Xen N  SQCDWLARLQ NGMVQNQYDP IRNGIQQGN- ACQAQALQHG LMTS-LHNGL PATTLSQMHT ———— -YQAMPNTRL ANQPHLMQAQ QMQQQQN—— ———— ——LQLHQS 2384
Dros N PSLPTSPTHI QAMRHATQQK QFGGSNLNSL LGGANGGGVV GGGGGGGGGV GQGPQNSPVS LGIISPTGSD MGIMLAPPQS SKNSAIMQTI SPQQQQQQQQ QQQQQHQQQQ QQQQQQQQQQ 2565

                                                                                    PEST-containing Region
hum N  APQPQSTCPP AVAGPLPTMY QIP———EM ARL-PSVAFP TAMMPQQDGQ VAQTILPAYH PFPASVGKYP TPPSQHSYAS SNAAERTPSH SGHLQGEHPY LTPSPESPDQ WSSSSPHSA- 2433
TAN-1  PPQPHLGVSS AASGHLGRSF LSGEPSQADV QPLGPSSLAV HTILPQ-ESP ALPTSLPSSL VPPVTAAQFL TPPSQHSY-S S-PVENTPSH QLQVP-EHPF LTPSPESPDQ WSSSSPHSNV 2520
Xen N  HQQQHHN-SS TTSTHINSPF CSSDISQTDL QQM—SSNNI HSVMPQ-DTQ IFAASLPSNL TQSMTTAQFL TPPSQHSY-S S-PMDNTPSH QLQVP-DHPF LTPSPESPDQ WSSSSPHSNM 2497
Dros N QQQLGGLEFG SAGLDLNG-F CGSPDSFHSG QMNPPS——I QSSMSG-SSP STNMLSPSSQ HNQQAFYQYL TPSSQHS—— ——GGHTPQH LVQTL-D-SY PTPSPESPGH WSSSSPRSN- 2671

hum N  SDWSDVTTSP TFGGAGGGQR GPGTHMSEPPHNN MQVYA                                                                    2471
TAN-1  SDWSEGVSSP PT———SMQ SQIARIPEAFK                                                                           2556
Xen N  SDWSEGISSP PT———SMQ PQRTHIPEAFK                                                                           2523
Dros N SDWSEGVQSP AANNLYISGG HQANKGSEAIYI                                                                         2703
```

FIG.17E

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/00825

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1; 435/6, 7.1, 69.1, 172.1, 172.3, 240.2, 240.21, 243, 320.1; 514/2, 44; 530/350, 387.1, 388.1; 536/23.1, 23.2, 23.4, 23.5, 24.1, 24.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GENETICS, Volume 126, issued November 1990, Xu et al., "*deltex*, a Locus interacting with the neurogenic genes, *Notch*, *Delta* and *Mastermind* in *Drosophila melanogaster*", pages 665-677, see entire document.	1-91
Y	WO, A, 92/19734 (ARTAVABUS-TSAKONAS ET AL.) 12 NOVEMBER 1992, see entire document.	1-91
Y	TRENDS IN BIOLOGICAL SCIENCES, Volume 17, issued April 1992, V. Blank et al., "NF-κB and related proteins: Rel/dorsal homologies meet ankyrin-like repeats", pages 135-140, see entire document.	1-91

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
02 MAY 1995

Date of mailing of the international search report
25 MAY 1995

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
BRIAN R. STANTON
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/00825

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TRENDS IN GENETICS, Volume 7, Number 11/12, issued November/December 1991, S. Artavanis-Tsakonas et al., "Choosing a cell fate: a view from the *Notch* locus", pages 403-408, see entire article.	1-91
Y	EUROPEAN JOURNAL OF BIOCHEMISTRY, Volume 208, issued 1992, K. Roemer et al., "Concepts and strategies for human gene therapy", pages 211-225, see entire document.	66-68, 81, 82, 84, and 86-91
Y	CHEMICAL REVIEWS, Volume 90, Number 4, issued June 1990, E. Uhlmann et al., "Antisense oligonucleotides: A new therapeutic principle", pages 544-584, see entire document.	81, 82, 84, 86-88, 90, and 91
Y	WO, A, 89/12690 (GARGAN ET AL.) 28 DECEMBER 1989, see entire article.	9, 10, 18, 21-26, 69, 70-80, 85, 90, and 91
Y	JOURNAL OF REPRODUCTIVE MEDICINE, Volume 37, Number 6, issued June 1992, E.M. Karson et al., "Prospects for human gene therapy", pages 508-514, see entire document.	66-68, 81, 82, 84, and 86-91

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/00825

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/00825

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 35/12; C07H 17/00; C07K 1/00, 14/00, 16/00; C12N 1/00, 5/00, 15/00; C12Q 1/00, 1/68; G01N 33/53

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/130.1; 435/6, 7.1, 69.1, 172.1, 172.3, 240.2, 240.21, 243, 320.1; 514/2, 44; 530/350, 387.1, 388.1; 536/23.1, 23.2, 23.4, 23.5, 24.1, 24.3

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

Databases: APS, Genbank, Biosis, CA, Medline, Embase
Search Terms: artavanis?/au; busseau?/au, diederich?/au; xu?/au; matsuno?/au; deltex; delta; delt?; notch; bind?; drosophil?; human; protein#; antibod?; monoclon?; polyclon?; cdc10; sw16; ankyrin; repeat#; glu (w) rich; sh3; sequence#; dna; cdna; genom?; genet?; pcasper; ha-dx; antisens?; hybridiz?; assay; cervical; breast; colon; melanoma; seminoma; lung; rna; malign?; bind?

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

I. Claims 1-14, 18-20, 90, and 91 drawn to purified Deltex proteins.

II. Claims 15-17 , drawn to chimeric proteins comprising a portion of Deltex proteins.

III. Claims 21-26, drawn to antibodies to Deltex proteins.

IV. Claims 27-49, 51-57, drawn to nucleic acids encoding Deltex proteins and associated vectors and host cells.

V. Claim 50, drawn to nucleic acids encoding chimeric proteins.

VI. Claims 58-65, 71-77, 79, 80, 82, and 83, drawn to pharmaceutical compositions comprising Deltex proteins and methods of treating disease using said compositions. (Note that claims 71-77, and 82 will be examined only to the extent that they read on the use of Deltex proteins as therapeutic agents.)

VII. Claims 66-68, 71-77, 82, and 84, drawn to pharmaceutical compositions comprising Deltex protein encoding nucleic acids. (Note that claims 71-77, and 82 will be examined only to the extent that they read on the use of Deltex encoding nucleic acids as therapeutic agents.)

VIII. Claims 69, 70-78, 82, and 85, drawn to pharmaceutical compositions comprising antibodies to Deltex proteins. (Note that claims 71-77, and 82 will be examined only to the extent that they read on the use of antibodies to Deltex proteins as therapeutic agents.)

IX. Claims 71-77, 81, 82, and 86-89, drawn to pharmaceutical compositions comprising Deltex antisense nucleic acids and methods of treating disease with said nucleic acids. (Note that claims 71-77, and 82 will be examined only to the extent that they read on the use of antisense nucleic acids as therapeutic agents.)

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

The compositions of the inventions of groups I-III do not share any special technical feature because they are drawn to divergent chemical compositions with differing properties. For example, the invention of groups I and II are drawn to proteins and that of group III to nucleic acids. Aside from their divergent chemical natures, these groups of compositions require separate analysis. For example, consideration of nucleic acids requires analysis of gene coding

regions and expression vectors and consideration of proteins require analysis of protein isolation, purification and function. The antibodies of the invention of group II are distinct from the proteins of group I because antibody compositions require analysis of means of producing specific protein binding reagents and uses thereof and such analysis is not required for consideration of structural proteins per se. Similarly, the inventions of groups IV-VI do not share a special technical feature because they are based upon the use of materially different compositions; proteins, nucleic acids and antibodies, respectively.

The proteins of the invention of group I do not share a special technical feature with the pharmaceutical compositions of the invention of group IV because said proteins may be used for the in vitro production of antibodies which does not involve use in the in vivo environment. Therefore, analysis of the proteins of group I does not require consideration of means of administration and assays for determination of efficacy as required for the analysis of the invention of group IV. Similarly, the antibodies of the invention of group II do not share a special technical feature with the pharmaceutical compositions of the invention of group VI because the latter invention requires analysis of the in vivo introduction of antibodies and because the antibodies of the invention of group II may be used in vitro.

The proteins of the invention of group I do not share a special technical feature with any of the methods of groups V-VII because said methods do not utilize said proteins and therefore consideration of said proteins per se is not required for analysis of any of said groups of methods.

The proteins of the invention of group I do not share a special technical feature with the method of the invention of group VIII because said method requires consideration of disease states and correlations between protein binding and said states and such consideration and analysis is not required for examination of proteins per se.

The antibodies of the invention of group II do not share a special technical feature with any of the methods of groups IV, V, VII and VIII because said methods do not utilize said antibodies and therefore consideration of said antibodies per se is not required for analysis of any of said groups of methods.

The nucleic acids of the invention of group III do not share a special technical feature with the pharmaceutical compositions of the invention of groups IV and VI because said compositions require consideration of in vivo use (see above) which is not required for analysis of nucleic acids per se and because said nucleic acids may be used in vitro. For example, said nucleic acids may be used to produce proteins.

The nucleic acids of the invention of group II do not share a special technical feature with the method of group VIII because said method does not utilize said nucleic acids and therefore consideration of said nucleic acids per se is not required for analysis of said method.

The nucleic acids of the invention of group III do not share a special technical feature with the methods and compositions of the invention of group VII because the latter invention involves the use of antisense nucleic acids which do not encode proteins, which regulate gene expression and which are designed for in vivo use. In contrast, the nucleic acids of the invention of group III encodes proteins (non-regulatory) and may be used for the in vitro production of proteins. Therefore, analysis of the nucleic acids of the invention of group III does not require the in vivo or regulatory considerations required for analysis of the invention of group VII. Similarly, the inventions of groups V and VII do not share a special technical feature because the former invention utilizes nucleic acids encoding proteins and the latter invention utilizes antisense nucleic acids that regulate gene expression.

The invention of group IV does not share a special technical feature with the methods of the invention of group VII because the latter methods utilize antisense nucleic acid therapeutic agents whereas the former utilize proteins. Consideration of the use of antisense nucleic acids requires analysis of selection of appropriate sequences and means of regulating gene expression and such analysis is not required for consideration of methods based upon protein therapeutic agents.

The invention of any of groups IV-VII does not share a special technical feature with the methods of the invention of group VIII because the former inventions utilize pharmaceutical compositions for in vivo treatment whereas the latter invention uses proteins for in vitro diagnostic. Therefore, the method of group VIII does not require consideration of in vivo methodologies.

The invention of group VI does not share a special technical feature with the invention of group VII because the former invention utilizes proteins and the latter invention utilizes antisense nucleic acids. Therefore, the analysis of each invention requires consideration of a distinct class of pharmaceutical.

Therefore, the separate inventions as listed above are not so linked by any single special technical feature so as to form

a single inventive concept within the meaning of PCT Rule 13.2. It is noted that Deltex proteins are used in all of the inventions of groups I-IV and VI-VIII, however, these proteins were known in the prior art and therefore do not represent a contribution over the prior art as defined by PCT Rule 13.2.